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東京帝國大學  
農學部紀要

第三卷



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OF THE

COLLEGE OF AGRICULTURE,

IMPERIAL UNIVERSITY OF TOKYO.

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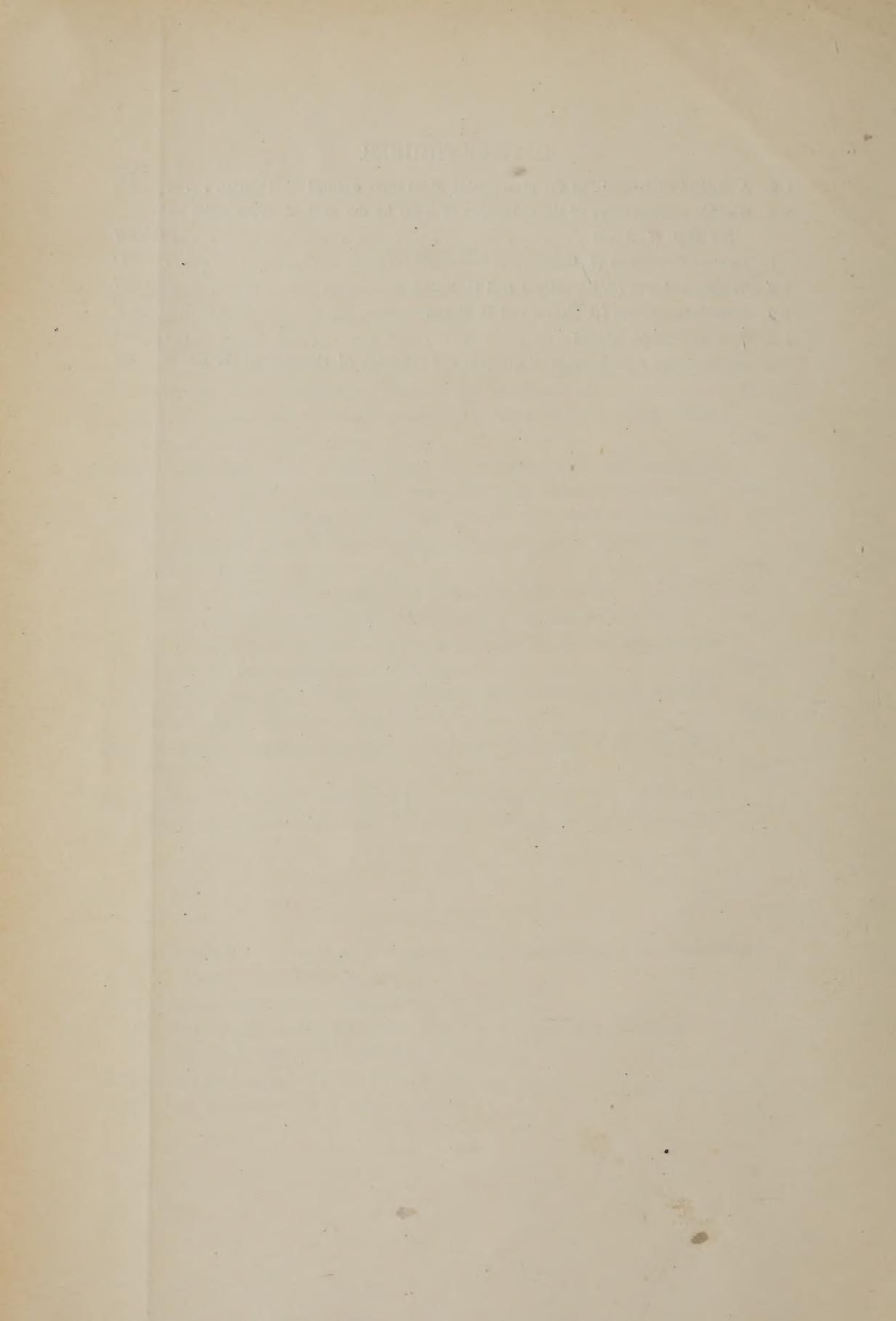
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All communications relating to this Journal should be addressed to the  
Director of the College of Agriculture.

**A Method of determining the Cross-section of an Open Channel  
by Ganguillet and Kutter's Formula.**

BY

**Seinen Yokota.**

---

**With Plate I—IV and two Figures in the Text.**

---

A problem that occurs when arranging the distribution of irrigation water is how to find the best form and size for an open channel, being given the discharge per unit time. The form of its cross section commonly adopted is a trapezoid with its sloping sides more or less inclined to the vertical.

To solve the problem, the bottom breadth is usually guessed at and the inclination of the sides fixed according to the nature of the earth in which the channel is to be excavated. The depth of water, being another variable, is also assumed at a convenient amount, and the cross-section A and the wetted perimeter p are then calculated. The former divided by the latter is called the hydraulic mean radius or depth. Then the mean velocity of flow is calculated by Ganguillet and Kutter's Formula, as being the most trustworthy among several. This mean velocity multiplied by the cross section gives the discharge per unit time. If this discharge does not give what is wanted, the bottom breadth or the depth of water is varied, and the above process repeated until the discharge comes out to be in agreement with requirements.

This tentative process is sometimes rather laborious, even with the aid of tables giving the coefficients of  $\sqrt{RJ}$  for several values of n, J & R in the Ganguillet and Kutter's Formula, viz.:—

$$(1) \quad v = \left\{ \frac{41.6 + \frac{0.00281}{J} + \frac{1.811}{n}}{1 + \left( 41.6 + \frac{0.00281}{J} \right) \sqrt{\frac{n}{R}}} \right\} \sqrt{\frac{R}{J}};$$

in which

$v$  = the mean velocity of water in feet per second,

$R$  = the hydraulic mean depth in feet,

$J$  = the slope of the water surface,

and  $n$  = coefficient of roughness of the wetted perimeter.

Hence, it has been my aim to avoid this repetitive process and to determine the cross section at once, when the quantity of discharge is given.

#### (a) The Cross Section of Minimum Wetted Perimeter.

If we introduce a relation between the bottom breadth  $b$  and the depth  $d$  so as to ignore one variable, the problem becomes far simpler and the cross section may be determined once for all. For this relation we may assume a cross section of minimum wetted perimeter, as causing the least frictional resistance or, in other words, as giving the maximum discharge for a given cross sectional area. This relation is given by

$$(2) \quad d = \frac{b}{2} \cot \frac{\theta}{2},$$

where  $\theta$  is the angle of inclination of the sides to a horizontal plane. So that

$$(3) \quad R = \frac{1}{4} b \cot \frac{\theta}{2}.$$

The quantity of discharge  $Q$  per second is equal to the sectional area  $A$  multiplied by the mean velocity per second. Therefore, from (1) and (3),

$$(4) \quad Q = \frac{k' k'' \sqrt{J} \left( k + \frac{1.811}{n} \right) b^3}{nk + \sqrt{k'b}};$$

in which

$$k = 41.6 + \frac{0.00281}{J}, \quad k' = \frac{1}{4} \cot \frac{\theta}{2}, \quad k'' = k' \times \frac{3 + \cot^2 \frac{\theta}{2}}{2}.$$

Using this notation, we have

$$(2)' \quad d = 2kb,$$

$$(3)' \quad R = k'b,$$

$$(5) \quad \begin{cases} \Lambda = k'' b^2, \\ p = \frac{k''}{k'} b. \end{cases}$$

The equation (4) gives  $Q$  in terms of the bottom breadth  $b$ . Hence,  $b$  is determinate when  $Q$  is given, and plotting  $Q$  as abscissa and  $b$  as ordinate, we get a graphic representation of the equation (4), giving  $b$  for a given  $Q$ . This  $b$  multiplied by  $2k'$  is equal to the depth  $d$ . Thus the cross section is determined for a given discharge.

When once the curves (4) are plotted on paper for several values of  $\theta$ ,  $J$  and  $n$ , they may be used thereafter and the laborious calculations involving the repeated application of Ganguillet and Kutter's Formula may be practically avoided, while the Formula acts as the basis of these curves.

Moreover, by the aid of these curves, the variations in  $Q$  due to that of  $n$ ,  $\theta$  or  $J$  may be traced. Conversely, the variations in the elements  $n$ ,  $\theta$  or  $J$  for a constant discharge may also be put in evidence. For intermediate values of  $n$ ,  $\theta$  or  $J$  other than those adopted below, the bottom breadth may be determined by a graphical interpolation.

To meet the various requirements occurring in practice, I have calculated the following sixty sets of curves for values of

$$\theta = 30^\circ, 45^\circ, 60^\circ \text{ and } 90^\circ;$$

$$J = \frac{1}{300}, \frac{1}{500}, \frac{1}{1000}, \frac{1}{3000} \text{ and } \frac{1}{5000};$$

$n = 0.020$  for rough rubble in cement, stone pitching,

$n = 0.025$  for rivers and canals in perfect order, free from stones or weeds, stone pitching in bad condition,

$n = 0.030$  for rivers and canals in good order.

Plates I, II, III and IV show these curves plotted for constant values of  $\theta = 30^\circ, 45^\circ, 60^\circ$  and  $90^\circ$  respectively.

In these plates, the scales for depths  $d (= 2k' b)$  are given along with the scales for bottom breadths  $b$ , so that  $b$  and  $d$  for a given  $Q$  may be read off at once. Logarithmic scales are used for  $Q$ .  $Q$  is in cubic feet per second and  $b$  &  $d$  in feet. These may be taken as cubic shaku (立方尺) per second and shaku (尺) resp., without sensible error.

## (b) Cross Section of Variable Depths deduced from that of minimum wetted perimeter.

Although the cross section of minimum wetted perimeter is the most economical from a theoretical point of view, it often happens that it is desirable on several practical considerations to have shallower or deeper sections than that determined theoretically.

For this purpose, we may take the cross section of minimum wetted perimeter as the standard and investigate the variations of its sectional area and wetted perimeter when it is changed into any arbitrary trapezoidal section while maintaining the same constant discharge.

The result of my investigation shows that, if we take for ordinate the ratio  $m (= \frac{d_1}{d})$  of the arbitrary depth to the standard and for abscissa the corresponding ratio  $x (= \frac{b_1}{b})$  of the bottom breadths, the curve for a constant discharge is a variable hyperbola, and varies very slightly for a constant inclination  $\theta$  (See Appendix).

These hyperbolas for  $\theta = 30^\circ, 45^\circ, 60^\circ$  and  $90^\circ$  are plotted in Plates I to IV respectively.

The ratios of the depths and the breadths referred to the standard section being thus given by a point on the curve, the cross section of any arbitrary depth may be determined as in the following examples.

Conversely, given a cross section,  $J & n$ , to find the  $Q$  or the velocity of flow, we may proceed as follows:—

We have

$$\frac{m}{x} = \frac{\frac{d_1}{d}}{\frac{b_1}{b}} = \frac{d_1}{d} \cdot \frac{b}{b_1}, \text{ a known number, say } F.$$

Therefore, at  $x=1$ , take  $m=F$  and join this point with the origin. The point of intersection of this line with the hyperbola is the corresponding point of the given section, and by dividing  $b_1$  by the corresponding  $x$ , we get the bottom breadth of the standard section, whence  $Q$  is read off from the curve (4).

This  $Q$  divided by the given sectional area is the mean velocity required.

Example 1. To find the standard section for  $Q=50$  cubic feet per second, when  $\theta=30^\circ$ ,  $n=0.03$  and  $J=\frac{1}{1000}$ . In Plate I, we read

$$\begin{aligned} b &= 1.75 \text{ ft.,} \\ d &= 3.27 \text{ ft.} \end{aligned}$$

Example 2. In the above example, if the depth of the channel is to be 2.5 feet, find the corresponding bottom breadth.

We have

$$\frac{d_1}{d} = \frac{2.5}{3.27} = 0.765.$$

Therefore, from the hyperbolæ,  $\frac{b_1}{b}=3.22$ , so that  
 $b_1=3.22 \times 1.75 = 5.63$  feet,

the corresponding bottom breadth.

Example 3. Find the quantity of discharge of a channel whose  $\theta=30^\circ$ ,  $n=0.03$ ,  $J=\frac{1}{1000}$ ,  $b_1=5.5$  feet and  $d_1=3$  feet.

We have

$$F = \frac{3}{5.5 \times 1.87} = 0.292.$$

Therefore, by drawing a line from the origin of the hyperbolæ through the point  $m=F=0.292$  at  $x=1$ , we read  $\frac{b_1}{b}=2.72$ .

Hence  $b = \frac{b_1}{2.72} = \frac{5.5}{2.72} = 2.02$  feet, and the corresponding quantity of discharge is read off from the curve, i.e.,  $Q=74$  cub. ft. per second.

Example 4. Find the mean velocity of flow for the channel in the preceding example.

$$\begin{aligned} \text{The sectional area} &= (b_1 + d_1 \cot 30^\circ) \times d_1 \\ &= (5.5 + 3 \times 1.73) \times 3 \\ &= 32.1 \text{ square feet.} \end{aligned}$$

Therefore, the mean velocity of flow =  $\frac{74}{32.1} = 2.3$  feet per second.

The above examples are for  $\theta=30^\circ$  throughout, but the processes of working are quite similar for other values of  $\theta$  in Plates II to IV.

In preparing the present paper, the author desires to acknowledge the kind assistance of a student (now a graduate) of the Agricultural College, who wishes to remain incognito and who worked out a mass of numerical calculations. His results were checked, corrected and plotted into curves by Mr. Naosaburō Kusakabe, Kōgakushi, and by the author.

Tokyo Imperial University,  
Tokyo, Japan, Dec., 1909.

## APPENDIX.

Using Ganguillet and Kutter's Formula, the discharge  $Q$  per second for any form of section is given by

$$(6) \quad Q = \frac{cR}{c' + \sqrt{R}} A ;$$

where  $c = \sqrt{J} \left( k + \frac{1.811}{n} \right)$  and  $c' = nk$ .

But  $R = \frac{A}{p}$ , so that

$$Q = \frac{c A^2}{c' + \sqrt{\frac{A}{p}}} ,$$

$$\text{or, } (7) \quad c^2 A^4 - 2cc' Q A^2 p + Q^2 (c'^2 p^2 - A p) = 0.$$

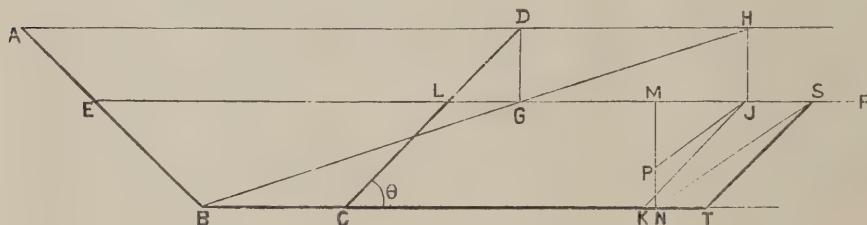
Taking the variation of  $A$  with respect to  $p$ , we have

$$\frac{\delta A}{\delta p} = \frac{2cc' Q A^2 - 2c^2 Q^2 p + Q^2 A}{4c^2 A^3 - 4cc' Q A p - Q^2 p} .$$

Now, inserting the relations (4) and (5), this becomes after reduction,

$$(8) \quad \frac{\delta A}{\delta p} = k'b \left( \frac{2nk + \sqrt{k'b}}{4nk + 3\sqrt{k'b}} \right) .$$

The variation of  $A$  with respect to  $p$  being thus determined in terms of  $b$  of the standard section, let



ABCD in Fig. 1 be the standard section for a given discharge  $Q$ , and EF the new water surface. From D draw DG perpendicular to EF, meeting it at G. Join BG and let it intersect AD at H. From H drop perpendicular HJ on EF meeting it at J. Then if we draw JK parallel to the side line DC, the area EBKJ = area ABCD.

From the intersecting point L of CD and EF, take LM = 2DL on EF. Draw the perpendicular MN from M on BC, intersecting it at N. From N on NM, take NP =  $\frac{\delta A}{\delta p}$ . Join PJ and draw NS from N parallel to PJ, intersecting EF at S.

Then the line ST drawn parallel to the side line DC gives with EB and BT the required cross section.

For, by the construction,  $PN = \frac{\partial A}{\partial p}$  and  $MN = d_1$ , the depth.

Also  $\frac{MS}{MN} = \frac{JS}{PN}$ , or  $\frac{MS}{d_1} = \frac{\frac{\partial A}{\partial p}}{\frac{\partial A}{\partial p}}$ . Therefore  $MS = \frac{\partial A}{\partial p}$ , and the relation is satisfied.

Hence, EBTS is the section with given depth  $d_1$  and discharge Q.

The construction is similar for deeper sections than the standard.

The above is a graphical solution of the problem.

Now from (2)' and (8),  $\frac{\partial A}{\partial p} = \frac{d}{2} \left( \frac{2nk + \sqrt{\frac{d}{2}}}{4nk + 3\sqrt{\frac{d}{2}}} \right)$ , so that  $\frac{\partial A}{\partial p}$  must always lie between  $\frac{1}{4}$  and  $\frac{1}{6}$  of the depth of the standard section.

Denoting the ratio  $\frac{\partial A}{\partial p} \div d$  by r, let us examine what sort of value it would attain for various values of nk and d.

The values of nk in our case are given by the following schedule:

Values of nk

$n =$	0.020	0.250	0.030
$J$			
$\frac{1}{300}$	0.85	1.06	1.27
$\frac{1}{500}$	0.86	1.075	1.29
$\frac{1}{1000}$	0.89	1.11	1.33
$\frac{1}{3000}$	1.00	1.25	1.50
$\frac{1}{5000}$	1.11	1.39	1.67

Taking the least and the greatest values of nk, the respective values of r are:

$$\text{Values of } r = \frac{\frac{\partial A}{\partial p}}{d} : -$$

	(nk=0.85)	(nk=1.67)	Mean
d = 0ft :	0.250	0.250	0.250
„ = $\frac{1}{2}$ ft :	0.224	0.235	0.230
„ = 1ft :	0.218	0.230	0.224
„ = 2ft :	0.210	0.225	0.217
„ = 4ft :	0.203	0.218	0.210
„ = 8ft :	0.197	0.211	0.204
„ = 18ft :	0.190	0.203	0.197.

Hence, for practical purposes, we may assume that the values of  $r$  for a given  $d$  remain constant throughout and are equal to the mean value cited above. The error affecting the result due to this assumption is small, as we will see later.

Now, let  $d_i = md$  be the depth of the required section. Then, in Fig. 1,

$$\frac{EG \times DG}{MN} = LJ.$$

$$\begin{aligned} \text{But, } EG &= b + (1+m) \cot \theta, d = d \left\{ \frac{1}{2k'} + (1+m)(2k' - \frac{1}{8k'}) \right\} \\ &= d \left\{ \frac{3-m}{8k'} + 2(1+m)k' \right\}; \\ DG &= (1-m)d; \end{aligned}$$

$$\& MN = md.$$

Therefore,

$$(9) \quad LJ = \frac{1-m}{m} \left\{ \frac{3-m}{8k'} + 2(1+m)k' \right\} d.$$

Also,

$$MJ = LJ - 2DL$$

$$\text{But } DL = (1-m) \operatorname{cosec} \theta, d = (1-m) \left( \frac{1}{8k'} + 2k' \right) d.$$

Therefore,

$$(10) \quad MJ = \frac{(1-m)^2}{m} \left\{ \frac{3}{8k'} + 2k' \right\} d.$$

Now,

$$JS = \frac{MJ \cdot PN}{PM} = \frac{MJ \cdot d \cdot r}{(m-r)d}, \text{ and substituting the value of } MJ \text{ from (10),}$$

$$(11) \quad JS = \frac{(1-m)^2 r}{m(m-r)} \left\{ \frac{3}{8k'} + 2k' \right\} d.$$

Since  $b_1 = b + LJ + JS$ , from the relations (2)', (9) & (11), we get after reduction,

$$\frac{b_1}{b} = x = \frac{\alpha - (2-m)\alpha r}{m-r} - m(\alpha - 1),$$

or,

$$(12) \quad (\alpha-1)m^2 + xm - (2\alpha-1)rm - rx - \alpha(1-2r) = 0,$$

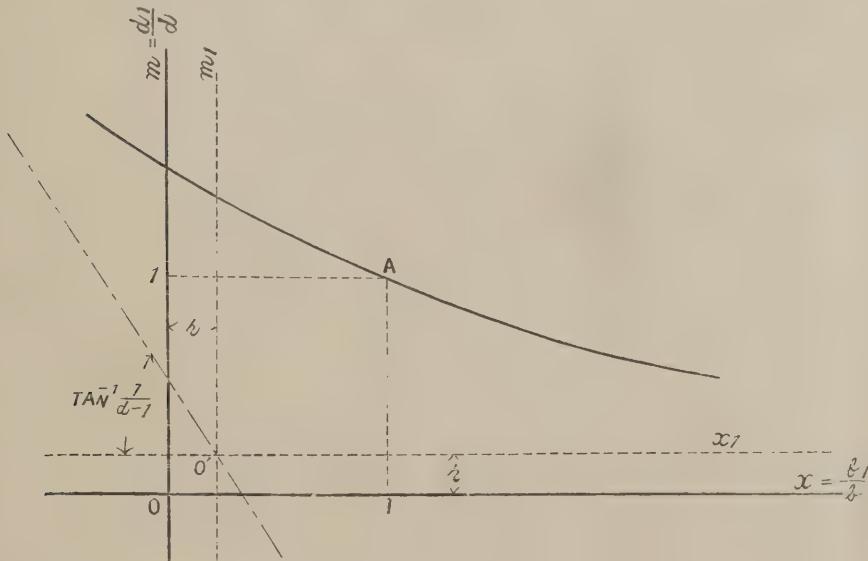
where  $\alpha = \frac{k''}{2k'}$ .

Transforming the origin to  $(r, r)$ , (12) becomes

$$(12)' \quad (\alpha-1)m_1^2 + x_1m_1 - \alpha(1-r)^2 = 0,$$

where  $m_1 = m - r$ ,  $x_1 = x - r$ .

This is a hyperbola with asymptotes  $m_1 = 0$  &  $(\alpha-1)m_1 + x_1 = 0$ , as in Fig. 2.



The family of curves (12) all pass through the point A (1,1), as is evident from the nature of our problem. If  $\theta$  be a constant, so also is  $\alpha$ . Then, the only variable parameter is  $r$  and it varies from  $1/4$  for  $d=0$  to  $1/6$  for  $d=\infty$ , so that the asymptotes displace parallel to themselves by their respective component amounts.

From (12), we find for a fixed value of  $m$ ,

$$(13) \quad \delta x = \alpha \frac{(m-1)^2}{(m-r)^2} \delta r.$$

Hence  $\delta x$  has always the same sign as  $\delta r$  and vanishes when  $m=1$ . For  $m>1$ , it increases with  $m$  from zero until it takes the value  $\alpha \delta r$  at  $m=\infty$ . For  $m < 1$ , it increases as  $m$  diminishes from zero to  $\infty$  when  $m=r$ .

In our cases, the values of  $\alpha$  are:

$$\begin{aligned}\alpha &= 4.23 & \text{for } \theta &= 30^\circ \\,, &= 2.21 &,, &= 45^\circ, \\,, &= 1.50 &,, &= 60^\circ, \\,, &= 1.00 &,, &= 90^\circ;\end{aligned}$$

while  $\delta r$  is less than 0.03.

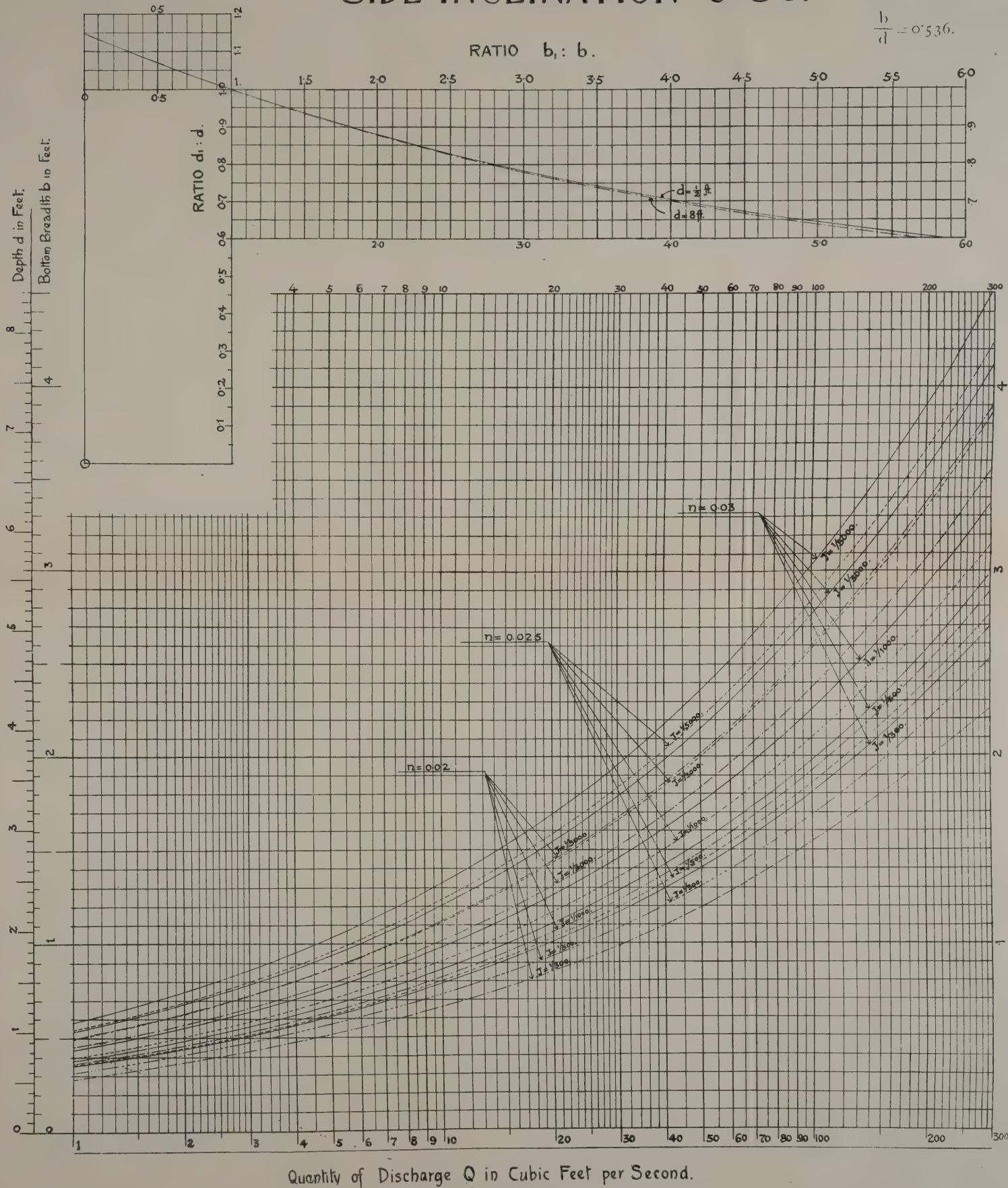
Hence we may safely leave out the correction  $\delta x$  for  $m>1$  in all cases. The correction is sensible when the channel becomes very shallow, so that the hyperbolae (12) are drawn for two values of  $d=1/2$  ft. and 8 or 10 ft. in Plates I to IV.

For intermediate values of  $d$ , the ratios  $\frac{b_1}{b}$  and  $\frac{d_1}{d}$  may be assigned by taking a point between the two hyperbolae.

SIDE INCLINATION  $\Theta = 30^\circ$ .

$$\frac{d}{b} = 1.866.$$

$$\frac{b}{d} = 0.536.$$

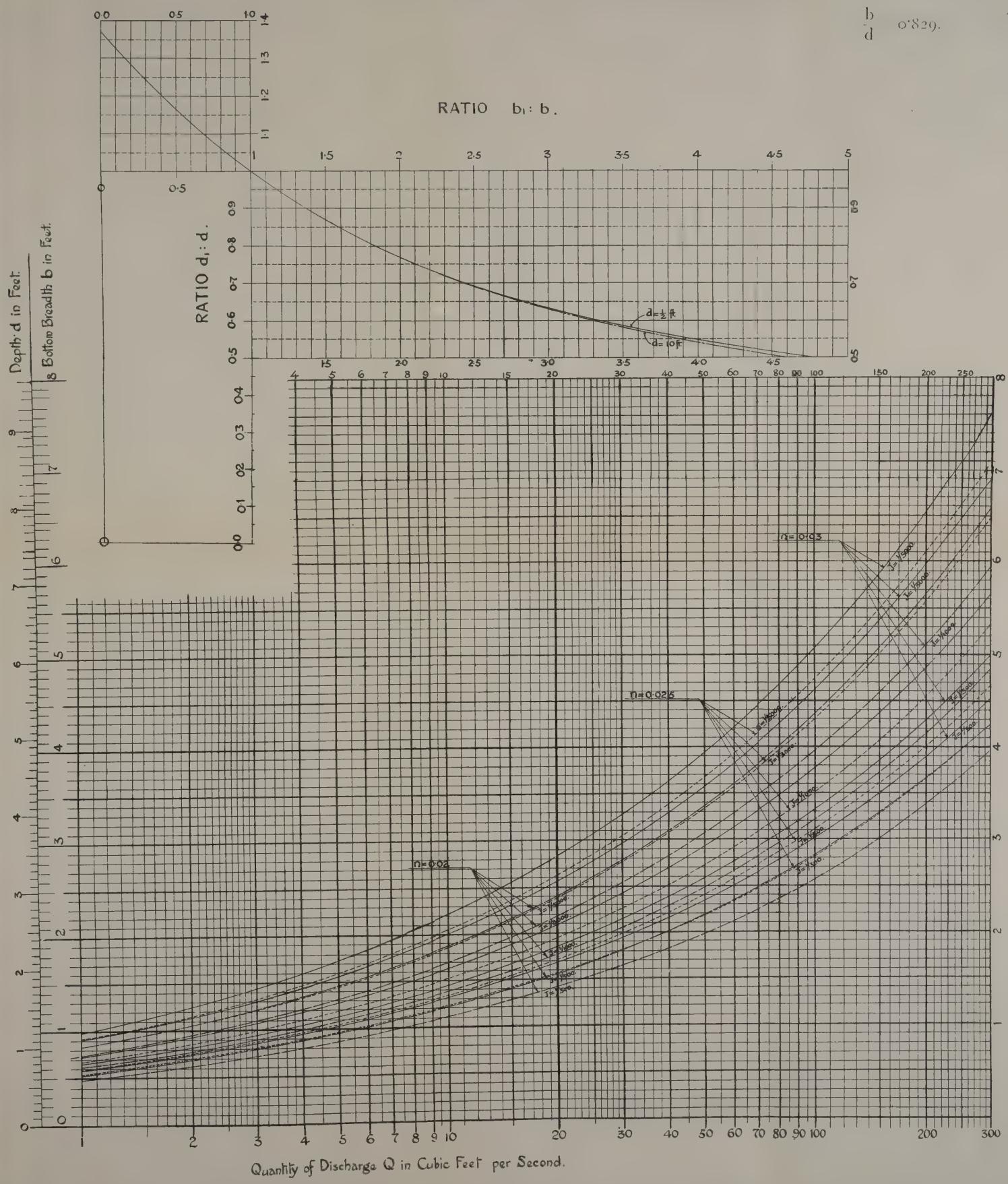
RATIO  $b_1 : b$ .



SIDE INCLINATION  $\theta=45^\circ$ .

$$\frac{d}{b} = 1.207.$$

$$\frac{b}{d} = 0.829.$$

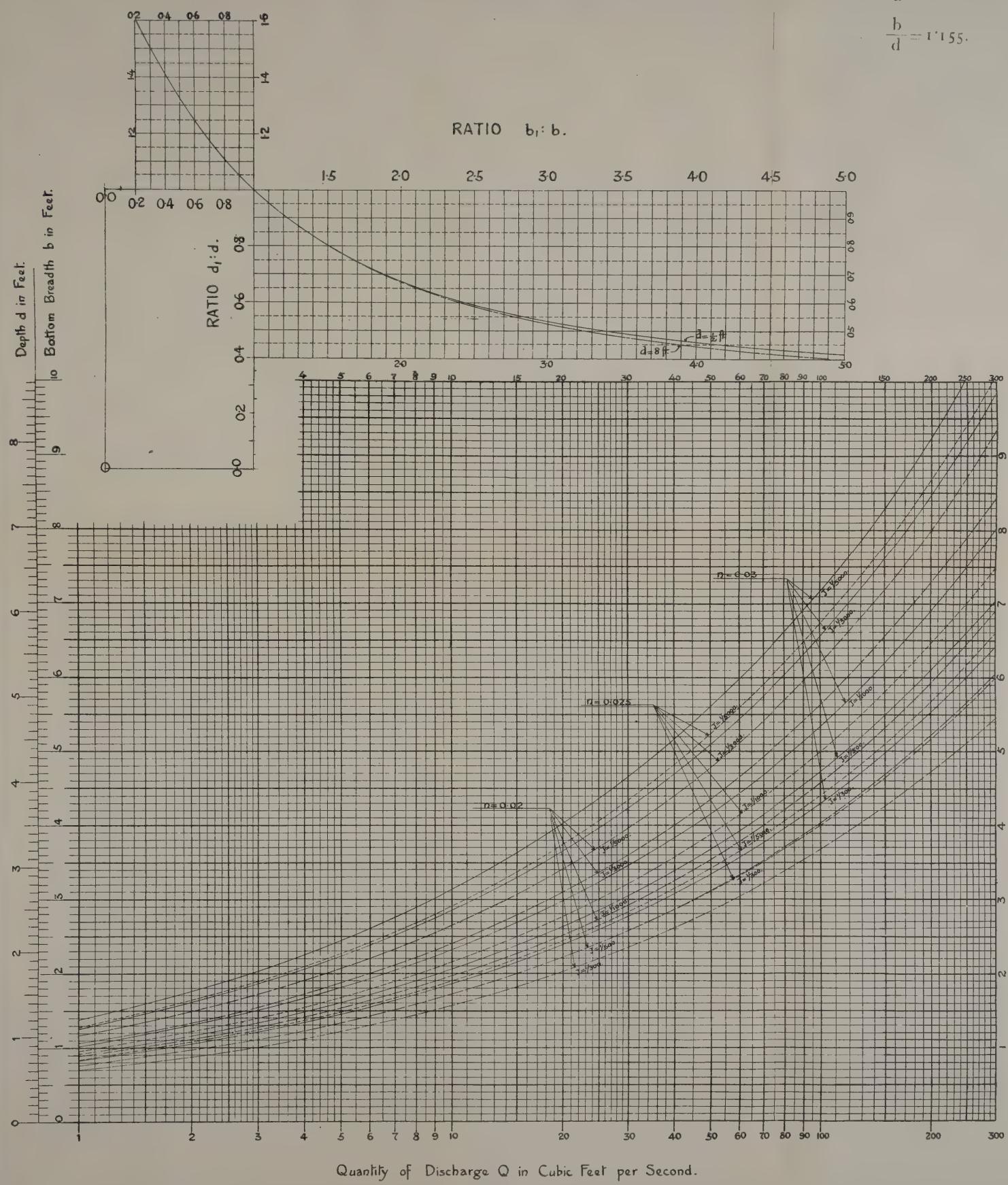




SIDE INCLINATION  $\Theta = 60^\circ$ .

$$\frac{d}{b} = 0.866.$$

$$\frac{b}{d} = 1.155.$$

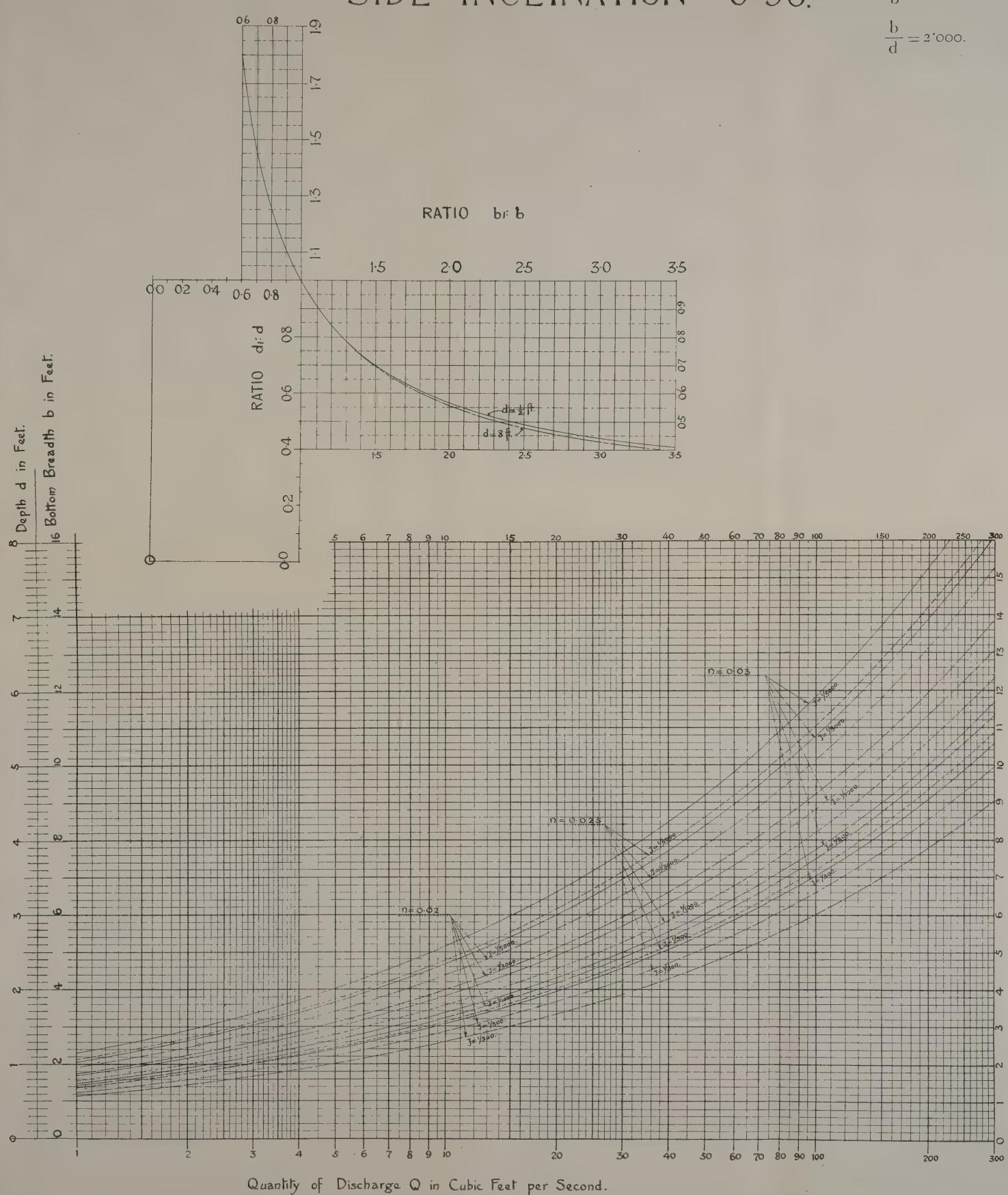
RATIO  $b_1 : b$ .Quantity of Discharge  $Q$  in Cubic Feet per Second.



SIDE INCLINATION  $\theta=90^\circ$ .

$$\frac{d}{b} = 0.500.$$

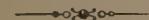
$$\frac{b}{d} = 2.000.$$





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All communications relating to this Journal should be addressed to the  
Director of the College of Agriculture.

# On the Classification of Cultivated Rice.

BY

S. Kikkawa.

College of Agriculture, Komaba, Tokyo.

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With Plates V—VIII.

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Several scientists have hitherto tried to classify rice. Among them we may mention BRETSCHNEIDER, ROXBURGH, GRIFFITH, LOULEIRO, HEUZE, SATO and INABU. But the classifications by these authors are either very simple or incomplete, mostly perhaps in consequence of the lack of samples at their disposal.

In 1884 YOSHIO TANAKA made a classification of Japanese rice, taking in consideration the characters of the grain. This classification is much more reasonable and perfect than those by the above mentioned authors.

F. KÖRNICHE in his work: "Die Arten und Varietäten des Getreides" mentioned a far more perfect classification of rice.<sup>1</sup> He obtained a good many samples from several countries but his classification too is based solely on the standpoint of the grain. To this classification I. INAGAKI proposed some additions in 1894.<sup>2</sup>

Sir GEORGE WATT, to whom the writer is much indebted for help and advice during the compilation of this work, examined more than four thousand samples of Bengal rice on the occasion of the international exhibition in Calcutta. In the Dictionary of Economic Products of India he gives three varieties of wild rice, viz: *rufipogon*, *bengalensis* and *abuen-sis*, but in dealing with the cultivated plants he ranges them first according to the localities of production and then subdivides each group according to the season of cultivation.<sup>3</sup>

1. Handbuch des Getreides. Band I: Die Arten und Varietäten des Getreides. S. 232-234.

2. INAGAKI: Researches on the rice-plant.

3. Dictionary of Economic Products of India Vol. V. p. 504 and 530-533.

In 1900, SETSUSABURO TANAKA, the adopted son of Y. TANAKA published his classification of rice, in which he took the following points into consideration:—

1. The character of the flowering parts of the plant.
2. Perennial character of the root stock.
3. Size of the stem and leaf and their colour.
4. Quantity of water required during growth.
5. Number of days for the growth.

His classification of cultivated rice is as follows<sup>1</sup>:—

- I. Long-glumed cultivated rice (*Oryza sativa*, L. var. *glandiglumis*, Del.).
- II. Common cultivated rice (*Oryza sativa*, L. var. *typica*).  
Sub-var. 1. Non-glutinous rice (*utilissima*, Kcke.).  
(A) Ordinary rice (*communissima*).  

$$\left\{ \begin{array}{l} (a) \text{ Awned.} \\ (b) \text{ Awnless.} \end{array} \right.$$

$$\left\{ \begin{array}{l} (a) \text{ Long-grained (*longior*, Al.)} \\ (b) \text{ Round-grained (*cyclina*, Al.)} \end{array} \right.$$

$$\left\{ \begin{array}{l} (\text{I}) \text{ Large-grained (*communis*, Kcke.)} \\ (\text{II}) \text{ Medium-grained (*media*.)} \\ (\text{III}) \text{ Small-grained (*minuta*, Presl.)} \end{array} \right.$$

These are sub-grouped into 7 according to the colour of the grain.

(1) Glumes and awn or the tip of the glumes coloured alike.

(2) Glumes and awn or the tip of the glumes differently coloured.

These are again subdivided into 15, according to the colour of the glume or awn.

(B) Scented rice (*moschata*).

(C) Violet coloured rice (*violacea*, Mag.).

1. Journal of the Scientific Agricultural Society No. 42, p. 84-130.

Sub-var. 2. Glutinous rice (*glutinosa*, Lour.).

- { (a) Awnless.
- { (b) Awned.
  - { (a) Long-grained.
  - { (b) Round-grained.
    - { (I) Large-grained.
    - { (II) Medium-grained.
    - { (III) Small-grained.

The further sub-classification is nearly the same as in the case of sub-var. 1 and is omitted here.

The author added that he was not satisfied with the above classification and hoped to improve it after further study; but the writer is very sorry to say, that S. TANAKA, to whom we are much indebted for his contribution to the rice-classification, is no more.

The writer has for many years taken much interest in the study of rice, and a few years ago had occasion to obtain numerous specimens of paddy from India, Burma, Siam, Ceylon and Java, in addition to those previously obtained from China and Korea, and of seeing a kind of wild rice growing naturally in Siam. These facts induced him to venture on a contribution to this line of agricultural science.

In classifying cultivated plants we should of course consider their morphological characters, which are constant, but at the same time we have also to examine those characters, which, though not very constant or fluctuating considerably, are sometimes yet very important for agricultural purposes. Now in dealing with the classification of cultivated rice we have first to determine the principles of division. From an agricultural point of view we have two principles of classification, one the cultivation, the other the utility of the grain.

### The Classification of Rice with regard to its Cultivation.

#### (1) Common or aquatic rice and upland rice.

This is the classification according to quantity of water required and is very familiarly known to farmers in rice-growing countries. Some kinds of upland rice may be descendants of true upland species, such as *Oryza granulata*, Nees (Figs. 1, 2 and 5) and *Oryza latifolia*, Desv. (Figs. 3 and 11), which according to Sir GEORGE WATT are still found in mountainous districts of India. They have perennial woody root stocks and leaves without air-chambers; but all the upland rices, so far as the writer knows them, cultivated in the chief rice-growing countries, have no specialities in morphological and histological characters. Sir GEORGE WATT places all upland cultivated rice under his variety *abuensis* and does not regard these as in any material way derived from *O. granulata* but considers *O. latifolia*, Desv. (= *O. officinalis*, Wall.) almost intermediate between *O. granulata* and *O. sativa*, from the standard of botanical structure. If, therefore, hybridization has contributed toward the production of any of the forms of the less aquatic rices, then he believes attention should be first directed to *O. latifolia*.

Its remarkable inflorescence, forming umbellate divisions, borne on long naked peduncles, are characters frequently met with in certain cultivated rices. But the variety *abuensis* (based on a wild rice found by I. F. DUTHIE on Mount Abu in Rajaputana) might quite well have originated all the races of cultivated upland rices without calling in the aid of either *O. granulata* or *O. latifolia* just as *O. coarctata*, Grif., (Figs. 4, 7, 8, 9 and 10), the wild rice of the Indus valley, of the lower Gangatic valley and of certain of the river basins of Burma, is quite unconnected with the aquatic cultivated rice.<sup>1</sup>

The writer has often experienced in several districts of Japan that some of the so called upland rices grow better and produce more in flooded

1. Sir GEORGE WATT's opinion, directly told to the writer.

field than in common field. Moreover, we can grow common rices on fields which are not watered and in many cases they yield fairly well.<sup>1</sup> Some varieties of rice in India are cultivated both on watered fields and on fields only flooded during germination.

From these facts we may imagine that most of the so called upland rices of the present day were formerly the same with the common varieties; and we can hardly draw a line between common or aquatic rice and upland rice. However, some of the latter, in consequence of repeated cultivation on dry fields for many years have acquired the character of enduring drought and can grow and yield fairly well on lands where common rices usually fail. Thus it is practically useful to make a reasonable classification of rice according to their water-requisite.

## (2) Early and late rice.

This classification is according to the difference of season of growth, or days of growth when sown at the same time.

Most Indian authors have assorted Indian rice into five seasonal groups as follows:

	Sown in	Transplanted in	Harvested in
Aus .....	April—May	Not transplanted.	July—Sep.
Aman .....	May	End of June to begin. of July.	November
Boro, kharif (Autumn)	June—July	July—Aug.	Sep.—Oct.
Boro, rabi (Spring) ....	Oct.—Nov.	Nov.—Dec.	May
Raydra .....	December	Not transplanted	Sep.—Oct.

In Siam there are two different groups of rice according to the season of growth, viz:

	Sown in	Transplanted in	Harvested in
Kao Bao .....	Jan.—Feb.	Feb.—Mar.	May—June
Kao Nak .....	June—July	July—Sep.	Dec.—Jan.

In Japan the time of sowing generally differs according to the district; and in the same district it may differ according to the variety,

1. Report of the Agricultural Experiment Station Vol. 15, No. 10.

but in the majority of cases all varieties in one district are sown at nearly the same time.

In this case, however, the time of ripening may of course greatly differ according to the variety. Those which ripen early are called early varieties and those which ripen later are either medium or late varieties. In the central part of Japan, the days required for the growth of each of these three groups are roughly shown as follows:

Early varieties .....	120—160
Medium varieties .....	150—180
Late varieties .....	170—200

Of course it is impossible to classify the rices of the world into such groups; even in one country, where climatic conditions considerably differ, such classification is practically useless; but in each district, within which the climate is nearly similar, this classification would hold good and be found very useful.

### (3) Giant rice.

The height of the common rice plant hardly exceeds two meters. The writer has not had many opportunities to carefully examine foreign rice plants in regard to their height; yet from such occasions as he has had of observing rice plants standing on fields in certain districts of India, Ceylon, Burma, Java and Siam, through which he passed, and a careful study of many Japanese rices, he has come to the above conclusion. The wild rice which is found in deep flooded places of tropical countries may attain the height of several meters. In November 1907 the writer found wild rice, *O. sativa*, densely growing in Klon Rangsit about ten miles distant from Bangkok, the capital of Siam. As usual after the rainy season of that country, the Klon, that is the canal, was filled with water about one to one and a half meter deep. The wild plant fixing its roots on the bottom of the canal, sends out its stems to the surface of the water, to which the stems naturally kept their position obliquely, because they were always forced to bend to the flow of water.

From the surface of the water the stem stood nearly upright about one meter high, including the ear. Thus the whole length of the plant measured generally from 2.5 to 3 meters (Figs. 12, 13 and 14). Having the natural character of growing higher with the rise of the water level, some cultivated rices become quite as tall as the wild ones. For example I may mention a rice cultivated in the district near Ayuthia, the ancient capital of Siam. In this district where water may often cover the land more than one meter deep, farmers cultivate varieties of rice which can grow safely out of the deep water and attain a height of two and half meters or more. A bunch of a giant rice, cultivated at the above mentioned district is shown in Figure 15. The specimen was sent by Prof. Dr. K. TOYAMA to our college when he was staying in Siam as an expert to the Government of that country. Sir GEORGE WATT says, that in India some of the Boro (or swamp rices) grow to a length of ten to fifteen feet, the harvest being made from boats, and this crop is of much value to the localities where produced, since the rice comes into season in May and June and thus lowers the price of the stocks.

Such extraordinarily tall rices are of special use in fields which may often be covered with deep water in consequence of river inundations or continuous heavy rain, and should better be grouped out of the ordinary ones. The writer calls those which are taller than two meters the "Giant-rice."

#### (4) Salt rice.

In some localities near the sea, rice fields may often become inundated or be artificially flooded with water mixed with a considerable quantity of sea water. In 1908, the writer got a small quantity of seed of so-called salt rice grown in Bombay Presidency, through the courtesy of Prof. GAMMIE of Poona, for the purpose of comparing its resisting power against the injurious action of sea-water, with that of three common rices of Japan. These rices were grown in a porcelain pot filled with the common soil of our college and were irrigated with an artificial sea water which contained the essential substances of the natural one in their average per-

centages, until its injurious action upon the plants were clearly seen.

In this case it was distinctly observed that the so-called salt rice had the strongest resisting power against the injury of the sea water among the rices planted together (Fig. 6). The salt rice is therefore quite worthy to be classified as a distinct group by itself.

But it may be repeated that *Oryza coarctata*, the wild rice of the margins of many of the larger rivers of India, though it luxuriates in brackish water, is unconnected with the cultivated salt rices.

#### (5) Tall and short rice.

Among the rices of the usual height, which hardly exceed two meters, as I have already stated, some are taller and some shorter. The height of Japanese rice plants ranges from 1 to 1.7 meter and in the majority of cases averages from 1.3 to 1.5 meter. The writer calls those taller than 1.7 meter tall rice and those shorter than 1 meter short rice. Such classification is of use in consideration of strong winds and space to be given to the plants in the field. According to the writer's examination of the Japanese rice plants, height has a distinct correlation with their tillering power and weight of the ear. In the years 1897-1900, the writer tried to test this correlation of many varieties, which he planted on a fairly good soil of the San-in Experiment Station, manured ordinarily and giving each seedling a space of 900 square centimeter. In their full maturity, the plants were harvested separately and completely air-dried and the number of stems and average weight of the ear of each plant were recorded. The result of the average of ten plants of each variety was as follows:—

Table showing correlation between length of stem and tillering-power.

(Average result of 4 years from 1897 to 1900)

Length of stem m.            m.	Average number of stems
0.882—0.939 (8 varieties) .....	19.0
0.970—1.030 (10     , ) .....	17.5
1.042—1.112 (10     , ) .....	14.3

Table showing correlation between length of stem and weight of ear.

(Result of the year 1900)

Length of stem m.            m.	Average weight of ear g.
0.619—0.832 (12 varieties) .....	2.14
0.840—0.895 (13     , ) .....	2.40
0.913—1.000 (13     , ) .....	3.19

There are a few rices, whose height is less than  $\frac{2}{3}$  meter. The writer calls such rices "dwarf rice." The dwarf rice, which has a low practical value is often grown for the sake of curiosity (Fig. 16).

#### (6) Awned and awnless rice.

This is one of the most notable differences in rice. The awn of rice is utterly useless to man and it gives a very disagreeable feeling to the workman when it comes in contact with him. The farmer therefore prefers awnless varieties, unless he has some special awned varieties, the valuable properties of which cannot be found among the awnless stocks. In ancient times when little care was taken of crops the awn might have been of some use for the protection of the grain against birds and other animals, and in the natural state of the plant it might have been more useful for the protection or as a means of distribution, but it is quite useless in the present conditions of agriculture. Thus most of the prevalent varieties in advanced centers of rice-culture are awnless. Sir GEORGE WATT observes that the general conclusion to be drawn from an analysis

of the cultivated forms of Indian rice is that progression in value is from the awned to the awnless and from the coloured to the colourless.<sup>1</sup>

Now, summing up the points of distinction discussed above for the classification of the rice in reference to its cultivation, they may be arranged as follows:—

(A) Aquatic rice.

- (a) Early rice. (b) Medium rice. (c) Late rice.

(I) Ordinary rice.

- (a) Tall rice. (b) Medium tall rice. (c) Short rice.

- (1) Awned. (2) Awnless.

(II) Special rice.

- (a) Giant rice. (b) Salt rice.

(B) Upland rice.

- (a) Early rice. (b) Medium rice. (c) Late rice.

- (a) Tall rice. (b) Medium tall rice. (c) Short rice.

- (1) Awned. (2) Awnless.

(7) The colour of the empty glume, glume, awn and tip of the glume.

The colours of the empty glume seen in Burman rice are white, light brown, brown, reddish brown and brownish purple; those of the glume are light yellowish white, which is the most common, brownish black, brown, dark brown, purplish brown, light ocher, ocher, yellow, buff, light buff, brownish yellow, light brownish yellow, light purplish brown, yellowish brown, drab and canary; those of the awn white, light brown, brown, reddish brown and yellow; and those of the tip of the glume are light yellowish white, which is the most common, brownish black, brown, dark brown, purplish brown, light ocher, ocher, light yellow, yellow, light brown, light brownish yellow, brownish yellow, blackish brown, yellowish brown, reddish brown and dark drab.

There exists no definite relation between each of the colours of the above mentioned parts of the unhulled grain, nor is there any definite

1. Dictionary of the Economic Products of India Vol. V. p. 505.

connection between the colour of the hulled grain and the colour of every part of the unhulled grain. But such colours may be made use of as marks of distinction of varieties.

(8) The colour of the stem and leaf.

The usual colour of the stem and leaf is green which turns greenish yellow towards maturity but there are a few rices, the stem and leaf of which show a dark violet colour. Such rices are found cultivated in oriental tropical countries and China, and in Japan only for the sake of curiosity, under the name of violet-rice, black-rice or crow-rice. The violet-rice contains in the epidermis of the plant a certain violet colouring matter which almost entirely covers the colour of the chlorophyl in the young stages, but becomes thinner towards maturity, the colouring matter being taken away by rain, etc. The peculiarity of the colour of such rice of course necessitates their being classified as a group by themselves and I. INAGAKI and S. TANAKA so esteemed them, that they placed the violet-rice as one of the largest divisions in the non-glutinous rice. The writer however does not consider it of such importance, because varieties of such rice are very rare and have no practical importance, nor do they show any sign of becoming important in future. He places the group among that having dark purplish brown husks, because the husk of the violet-rice has this colour.

(9) Long-glumed rice.

The empty glumes of the common rice are small and their length is usually less than one-third of the flowering glumes. A few rices however have very long empty glumes, the length of which mostly exceeds that of the flowering glumes. This rice is called the long-glumed rice. The long-glumed rice is cultivated in oriental tropical countries, South America, China and Japan. S. TANAKA laid much stress upon the investigation of it. He supposed it to be a descendant of an original long-glumed form, which has already passed away. Thus he classified it as one of the greatest divisions among the cultivated rice. He said that

some farmers in Japan believe that long-glumed rice is generally more resistible against the injury of winds than common rice. This may be a fact but at the same time they are generally inferior in the quality of grain, and have never taken an important position in rice-culture anywhere, and will doubtless pass out of use, as agriculture advances. The writer does not consider them as an important group and places them along those assorted according to the colour of husk.

(10) Double rice.

Some varieties of rice are said to contain more than one ovary in a spikelet. Col. D. PRAIN gave to such rice the Latin name "plena." Sir GEORGE WATT says, that a cultivated rice exists in Chittagong with two to seven ovaries, and he places the plena among the four varieties of *O. sativa*<sup>1</sup>. The writer has had no opportunity to examine any specimen of such rice, nor has he read any detailed description of it.

For further classification of rice, such morphological characters as the form of the panicle and the colour of the stigma should be considered.

### The Classification of Rice with regard to the Utility of the Grain.

(1) Non-glutinous and glutinous rice.

This is a distinction noticed from ancient times. There are no remarkable differences in the morphological characters between the groups, but glutinous rice have generally more tender stems and leaves than those of non-glutinous rice, so that their straw is more valuable for many purposes than that of the other. The principal differences between the two classes are seen in the character of their grain. The grain of the glutinous rice when fully ripe and well dried becomes quite opaque and

1. WATT'S Commercial Products of India, p. 824.

shows a chalky white colour, but when steamed it becomes much more transparent and shows more viscosity than that of the non-glutinous kind.

In 1860 A. GRIS discovered that starch of some rice shows a reddish-brown colour, when zinc-chloride solution of iodine is added to it.<sup>1</sup> Afterwards F. KŒRNICKE found that such reaction always appears with the starch of glutinous rice. He discovered too, that iodine colours the starch of glutinous rice yellowish brown, while it turns the colour of starch of non-glutinous rice into violet.<sup>2</sup> U. KREUSLER and W. DAFERT determined by chemical investigation that a part of starch in the glutinous grain is replaced by sugar and dextrine.<sup>3</sup> According to J. SHIMOMYAMA, the endorperm of the glutinous rice contains only a small percentage of common starch, but it contains a considerable percentage of soluble starch and dextrine besides some maltose.<sup>4</sup>

As far as the writer knows, the glutinous rice is cultivated in India, Burma, Siam, Java, China and Japan (Korea and Formosa included). But in no country does it take the place of common food of the people and it is chiefly used as a kind of sweets. In Japan it is only occasionally used as "kowa-meshi," a kind of "meshi," which is the chief daily food of the nation and is prepared by boiling the non-glutinous rice with water. Thus the use of the glutinous rice is quite different to that of the non-glutinous. The classification of the rice into these two groups is, therefore, of first importance.

## (2) Long-grained and short-grained rice.

Although there are great variations in shape of rice-grain (hulled), such classification is very important. The words long and short show the relative difference in length and breadth of the grain, thickness never exceeding breadth. The writer calls those grains, which have the length more than twice of its breadth as long-grain and those with length

1. Bulletin de la Societe botanique de France 7, 1860, p. 876.

2. Arten und Varietäten des Getreides S. 244-225.

3. Landwirtschaftliche Jahrbücher, 1884, S. 767-771.

4. Journal of Tokyo Igakkai, Vol. I, No. 2-Vol. II, No. 2.

less than twice the breadth as short grain, which S. TANAKA called the round-grain. The long-grained rice is much more easily broken than the short-grained in operations of hulling, whitening and polishing and rice mixed with broken grains cannot be boiled uniformly. It is quite natural that the long-grained rice, which is much valued among some people as table rice, is very expensive, if it be prepared free from broken grains.

The grain of some rice is extraordinarily long, its length exceeding thrice the breadth. The writer calls such rice "slender-grained."

### (3) Large, medium and small grained.

The classification according to the size of the hulled grain too is very useful, firstly because the size has an important connection with the taste, secondly because the rice may be differently used according to its size. Moreover, in districts of comparatively cool climate, rice of large grain can never be successfully cultivated. The size of the grain is most accurately expressed with its volume. For the purpose of measuring the grain-volume, the writer recently constructed a volumenometer, with which the average volume of rice-grains can be quickly measured. The size of the rice-grain may also be expressed by its three dimensions, and as the thickness of the rice-grain of a certain shape does not show so considerable differences as its other two dimensions, the measurement of the length and breadth can show the relative size of the grain with tolerable accuracy. The expression of a relative size of rice-grain by the two dimensions is useful for practical purposes, because the measurement is easily done with a simple measure or a micrometer and it shows at the same time which shape the grain belongs to.

Now, in determining the three relative sizes of the rice-grain by its length and breadth, the writer examined rice-grains of Burma for the standard, because Burma is the most extensive rice exporting country in the world and produces rice of almost all sizes as well as all shapes, and he obtained samples of paddy of a considerable number of varieties of the country by the favour of Mr. MACKENNA, the Director of Agriculture of Burma, who on his request kindly

collected and sent him these samples, which amounted to one thousand and twenty four. The writer wishes here to express his sincere thanks to him for his kindness.

The classification of Burman rice by the writer according to the shape and size of the hulled grain is as follows:—

(L.=Length; B.=Breadth.)

( I ) Slender grained.  $\frac{L}{B} > 3$

- (1) Large. L. over 7.5 m.m. and B. over 2.5 m.m. or  $L \times B. > 18.75$  s.m.m.
- (2) Medium. Those belonging neither to the large nor to the small grain.
- (3) Small. L. under 7.0 m.m. and B. under 2.2 m.m. or  $L \times B. < 15.40$  s.m.m.

( II ) Long grained.  $3 > \frac{L}{B} > 2$

- (1) Large. L. over 6.5 m.m. and B. over 3.0 m.m. or  $L \times B. > 19.5$  s.m.m.
- (2) Medium. Those belonging neither to the large nor to the small grain.
- (3) Small. L. under 6.0 m.m. and B. under 2.5 m.m. or  $L \times B. < 15.0$  s.m.m.

(III) Short grained.  $2 > \frac{L}{B}$

- (1) Large. L. over 6.0 m.m. and B. over 3.0 m.m. or  $L \times B. > 18.0$  s.m.m.
- (2) Medium. Those belonging neither to the large nor to the small grain.
- (3) Small. L. under 5.5 m.m. and B. under 3 m.m. or  $L \times B. < 16.5$  s.m.m.

The important physical properties of the unhulled and hulled grain of 971 varieties of Burman rice, which the writer has examined with the help of K. TANIGUCHI, then assistant to our laboratory, and classified according to the above standards, are shown in the table in the appendix.

The following table shows how the product of the length by breadth of the hulled grain harmonizes with its real volume, in average of each form of Burman rice grains, of which the length, breadth and volume were measured.

		No. of varieties averaged	Length m.m.	Breadth m.m.	Product of L.×B.	Volume of 1000 grains c.c.	Quotient of L.×B.÷vol.
Slender	Large	24	7.866	2.517	19.742	18.46	1.069
	Medium	71	7.274	2.321	16.805	15.32	1.097
	Small	7	6.981	2.137	14.957	13.63	1.097
Long	Large	47	7.264	2.980	21.602	20.76	1.041
	Medium	103	6.539	2.606	17.618	16.54	1.065
	Small	33	5.566	2.304	12.847	11.25	1.142
Short	Large	25	5.980	3.146	18.821	18.60	1.012
	Medium	44	5.730	3.027	16.879	16.40	1.029
	Small	18	5.124	2.883	14.765	13.87	1.065

#### (4) Common coloured and specially coloured.

The most prevalent colour of the rice grain is white which varies from a chalky white as usually seen in glutinous rice to a translucent waxy white, common in non-glutinous rice, sometimes with pale yellowish or grayish tint. Other colours found in the rice-grains are brownish red, whitish brownish red (*terra cotta* colour of Funk's standard Dictionary), purplish-black and pale-green. Brownish red colour is often found in non-glutinous rice, whitish brownish red colour sometimes in glutinous rice; purplish black colour is not seldom in the glutinous rice of tropical Asia.

The colour of the rice-grain is contained only in the pericarp of the grain and may be taken off with the bran, by the process of whitening. But the rice-grain has as a rule certain depressed lines lengthwise on its body and portions of skin in the lines often remain after the process of whitening, and consequently in case of specially coloured rice the remaining portions of skin may give a specially ugly appearance to the whitened rice. To get rid of the colour entirely much labour is required and at the same time a considerable percentage of the farinaceous portion of the grain should be lost. Thus it is quite right that specially coloured rices are considered inferior to the white one. The classification of rice according to their colour of grain is, therefore, of considerable importance.

## (5) Scented rice.

The newly harvested rice-grain and straw have a certain peculiar smell, which becomes gradually less and less and finally insignificant. Some varieties of non-glutinous white grained rice, however, preserve for many months that smell, which is considerably stronger than that of the ordinary ones. Such rices are called "scented." The scented rice is cultivated in oriental tropical countries and China, and rarely only in Japan. It is used for the sake of the smell by those people who have a special taste in this direction. Europeans as well as Japanese usually regard the smell as objectionable and compare it to the smell of mice. According to Sir GEORGE WATT, one of the most expensive rice of India, is that grown at Shait-Khan opposite to the fort of Bara about nine miles southwest of Peshawar. It is said to be only grown in a few fields and before the conquest of the Sikhs, the Cabol Sirdars had agents to watch the fields in order that none might be removed. It is a rice with pure white thin grains which are highly scented. The scented rice certainly deserves to be grouped by themselves, but their production is trifling, their demand being very limited.

Summing up the points of distinction with regard to the utility of the grain, they may be arranged as follows:—

## (A) Non-glutinous rice.

(I) Slender-grained. (II) Long-grained. (III) Short-grained.

(1) Large-grained. (2) Medium-grained. (3) Small-grained.

(a) Common-coloured.

(a) Ordinary. (b) Scented.

(b) Specially colored.

## (B) Glutinous rice.

(I) Slender-grained. (II) Long-grained. (III) Short-grained.

(1) Large-grained. (2) Medium-grained. (3) Small-grained.

(a) Common coloured. (b) Specially coloured.

## (6) The shape of the hulled and unhulled grains.

Among the above mentioned forms of rice-grains, there are various shapes, of which the writer distinguishes six main types in Burman rice, which are shown as A, B, C, D, E and F respectively in case of unhulled grains and as a, b, c, d, e and f in case of hulled grains in the classified table (Fig. 17).

The varieties representing these shapes are as follows:

Variety	District	Class	Shape of	
			Unhulled grain	Hulled grain
Baw-yoot	Maubin	Non-glut. Short, Large	A	a
Letywezin Samalawe	Maubin	Non-glut., Long, Large	B	b
Dawe Kaub-yin	Pyapon	Non-glut., Slender, Large	C	c
Nyaing-gaing	Sandoway	Glut., Slender, Large	D	d
Ahpoyochaw	Prome	Non-glut., Slender, Medium	E	e
Byat	Thaton	Non-glut., Long, Large	F	f

The shape of a hulled grain generally coincides with that of the unhulled grain of the same variety; but exceptions are not seldom. With regard to the heredity of the shape little is known; but as the shape has some connections with processes of whitening and taste, such distinction is useful.

## (7) White abdomened rice.

That side of the rice-grain in which the embryo is situated is called the ventral side. In the middle part of the ventral side of the non-glutinous rice-grain there exists usually a white or chalky looking portion. This white portion we call the "Harajiro," i.e. abdominal white. In most cases the abdominal white exists along the edge of the ventral side and extends more or less toward the center of the grain, but in some varieties it exists completely surrounded by ordinary waxy texture and imparts a dull white appearance to the grain. In the latter cases the writer calls the abdominal white "Shiratama-typed," because Shiratama, one of the most famous varieties of Japanese rice, possesses always such abdominal white and from this fact the variety name Shiratama was derived.

The abdominal white is almost always found in large grains belonging to long-grained and short-grained varieties. I. INAGAKI detected that the white abdomened rice-grain has a lower specific gravity, is more easily broken by pressure and the chalky coloured portion absorbs liquids more rapidly than the normal portion. Moreover he observed that the abdominal white exists farthest from the vascular bundle of the grain, that it appears at the germination of the grain, which has been entirely free from it before the germination, that rice not fully matured have mostly abdominal white and sometimes that portion where it exists is slightly depressed. He explained, thus, that the abdominal white is nothing but a portion of rice-grain, where spaces between starch-grains are not filled up with albuminous substances<sup>1</sup>. U. SUZUKI and K. Aso detected by chemical analysis that the chalky portion of the rice-grain has a lower percentage of albuminoides than the normal portion.<sup>2</sup> S. TANAKA expressed nearly the same opinion as I. INAGAKI and he added that the abdominal white results from insufficient supply of nutritive matters in the ripening time, or from abnormal arrangements or improper proportion of the accumulated substances in the grain.<sup>3</sup>

In examining Burman rice the writer observes that the abdominal white generally occurs only slightly or is entirely absent in slender grains, while it is often conspicuous in short ones, so that its magnitude seems to some extent to be proportionate to the breadth of the grain.

We may safely say that the abdominal white shows fluctuation according to climate, weather and methods of cultivation, but its heredity is not determined yet. It is, however, useful to mention the degree of its existence in the grain, because it has an influence upon the quality of the grain.

A similar difference of texture in the grain certainly exists in the glutinous rice too, but as the endosperm of the glutinous rice is generally

1. I. INAGAKI: Researches on the rice-plant.

2. Journal of the Scientific Agricultural Society, 1901, No. 47, p. 14.

3. Journal of the Scientific Agricultural Society, No. 42.

almost uniformly chalky coloured it is impossible to distinguish the abdominal white in the glutinous rice by its outward appearance and therefore we never speak of it with this group of rice.

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## A P P E N D I X.

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### BURMAN RICE,

Measured and classified according to the shape and size of the grain and described.

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### Abbreviations.

Abd.	Abdomen or abdominal.
b.	Brown or brownish.
bl.	Black or blackish.
com.	Common.
cons.	Conspicuous.
dar.	Dark.
g.	Grey or greyish.
gr.	Green.
l.	Light.
med.	Medium.
o.	Ocher.
p.	Pale.
pur.	Purple or purplish.
r.	Red or reddish.
sl.	Slight.
t.c.	Terra cotta.
w.	White.
y.	Yellow or yellowish.

## NON-GLUT

## SLENDER-

## L A R

Variety	District	Unhulled grain										
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Empty glumes	Clumes	Awn	Tip of glumes	Weight of grains per 100	Specific gravity
Bangauk	Tharrawaddy	10.26	3.17	2.24	0	C	w.	com.		l.b.	3.417	1.2171
Bangauk Saba	Pyapon	10.28	2.94	2.16	0	C	w.	com.		l.b.	3.446	1.1925
Bankouk Kouk-lat- myo	Bassein				0	C	w.	com.		l.b.		
Dawe-Kaub-yin	Pyapon	9.87	2.91	2.10	0	C	w.	com.		l.b.	3.645	,
Emata	Pyapon	10.28	3.03	2.23	0	C	w.	com.		l.b.	3.478	1.2127
Etmatat	Myaungmya				0-5	C	w.	com.	w.	com.		
Nat-Aw-za	Thaton	9.81	2.94	2.15	0	C	w.	com.		com.	3.342	1.2546
Palaung-Hmwe	Bassein				0	C-D	w.	o.		y.		
Shwe-Thwe	Pegu	10.12	3.05	2.22	0	C	w.	com.		l.b.	3.576	1.2011

## M E D

Ahpyechaw	Prome	9.44	2.81	2.12	0	E	w.	com.		com.	2.908	,
Aule	Bassein	10.13	2.84	2.11	0	C	w.	com.		com.	3.050	
Bangok	Akyab	10.14	3.06	2.25	0	C	w.	com.		l.b.	3.161	1.2103
Ematha	Prome	9.53	2.84	2.10	0	D	w.	l.o.		com.	2.420	
Hinemyi	Maubin				0	E	w.	com.		com.		
Kauk Lat Sawbwashe	Sandoway				0	C-E	w.	com.		com.		
Kauk-sal-Shatme	Sandoway				0	D	w.	com.		com.		
Kauk-thwe-hyu	Toungoo	9.87	2.90	2.07	0	C	w.	com.		com.	2.692	
Kauk-thwe-pyi Kyon Tee-ma	Maubin				0	C	w.	com.		com.	2.765	
Kawk-yin	Myaungmya	9.79	2.67	2.11	0	C-E	w.	com.		com.	2.814	
Kunbwegyi	N. Arakan				0	E	w.	com.		com.	2.971	
Kun-wa-yin	Tharrawaddy				0	C	w.	y.		l.y.		
Ky-Maung Kauk-yin-Myo	Bassein				0	C-E	w.	com.		com.		

## INOUS RICE.

## GRAINED.

G E .

N.-G., S., L. &amp; M.

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of 100 grains c. c.	Weight of 100 grains g.	Specific gravity	
8.03	2.66	1.91	3.02	1.39	c	com.	sl.	1.89	2.710	1.4339	793
7.69	2.52	1.89	3.05	1.33	c	com.	0	1.94	2.760	1.4227	801
7.82	2.59		3.02		c	com.	sl.				
7.73	2.47	1.91	3.13	1.29	c	com.	sl.	2.06	2.920	1.4175	801
7.65	2.55	1.95	3.00	1.31	c	com.	0	1.89	2.800	1.4815	805
7.50	2.50		3.00		c	com.	med.	1.83	2.580	1.4098	
7.68	2.46	1.84	3.12	1.33	c	com.	med.	1.89	2.676	1.4134	800
7.93	2.41		3.29		c	com.	0				Glumes lighter colored at base.
7.78	2.55	1.94	3.05	1.31	c	com.	0	2.03	2.819	1.3887	788

## I U M .

7.10	2.30	1.83	3.09	1.26	e	com.	sl.	1.51	2.184	1.4464	751
7.76	2.21	1.87	3.51	1.18	c	com.	med.		2.416		790
7.38	2.41	2.03	3.06	1.18	c	com.	sl.	1.77	2.532	1.4305	801
7.48	2.36	1.82	3.17	1.29	d	com.	0	1.34	1.908	1.4239	793
7.25	2.33		3.11		e	com.	0				
7.45	2.22		3.36		c-e	com.	0	1.50	2.200	1.4667	
7.82	2.49		3.14		d	com.	0				
7.17	2.32	1.79	3.09	1.29	c	com.	sl.	1.60	2.300	1.4375	
6.93	2.31		3.00		c	com.	0	1.55	2.176	1.4039	787
7.02	2.34	1.79	3.00	1.31	c-e	com.	sl.	1.43	2.108	1.4741	
7.12	2.37		3.00		e	com.	0	1.66	2.326	1.4012	783
7.10	2.31		3.07		c	com.	0	1.51	2.144	1.4199	
7.32	2.31		3.17		c-e	co n.	med.	1.60	2.284	1.4275	Glumes oft. b. colored between veins.

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of				Weight of 100 grains g.
							Empty glumes	Glumes	Awn	Tip of glumes	
Kyettu-ywe	Myaungmya				0	C	w.	com.		com.	
Kywe Nive	Sandoway				0	C-E	w.	b.		l.b.	
Lon-thwe Saba	Myaungmya	9.43	2.76	2.09	0	C	w.	com.		com.	2.772
Mi-kauk	Myaungmya				0	C	w.	com.		com.	
Moung-kaung	N. Arakan				0	E	w.	com.		com.	
Nga-Pyu-gale	Kyaupkyu				0	E	w.	com.		com.	
Ngwe-Moung	Myaungmya	9.78	2.85	2.05	0	C-E	w.	com.		com.	2.622
Ngwe-Moung	Kyaupkyu				0	C	w.	com.		com.	
Nwe-Maung	Sandoway				0	C-E	w.	com.		com.	
Palaung Kauk-yin Myo.	Bassein				0	E	w.	b.		l.b.	
Ralsun	N. Arakan	9.69	2.82	2.05	0	E	w.	com.		com.	2.722
Saw Bwa Kaukyin	Pyapon	9.52	2.84	2.10	0	C	w.	com.		com.	2.931
Saw Bwa	Sandoway				0	C-E	w.	com.		com.	
Shwe-thwe-mwe	Myaungmya	9.49	2.76	2.03	0	C	w.	com.		com.	2.566
Shwezwe	Toungoo	9.44	2.84	2.13	0	F	w.	com.		com.	2.954
Sut-mee	Sandway				0	C	w.	com.		com.	
Taungbaw Kaukyin	Pegu				0	E	w.	com.		com.	
Taungbzan	Henzada				0	C	w.	com.		com.	
Taung-deik-pan	Myaungmya				0	C	w.	com.		com.	
Taung-daik-pan	Henzada				0	C-E	w.	com.		com.	2.507
Taung Deib-pan	Tharrawaddy				0	C	w.	com.		com.	
Taung-kayin	Kyaupkyu				0	C	w.	com.		com.	
Taung Taikpa Myathla-Chaung	Maubin				0	C	w.	com.		com.	
Taung-Teikpan	Tharrawaddy				0	C	w.	com.		com.	
Tedawmo Kyauk Lat	Sandoway				0	E	w.	com.		com.	
Taung-yo Byu	Kyaupkyu				0	C-E	w.	com.		com.	
Yahaing	Myaungmya				0	E	w.	o.		com.	
Yahaing	Thaton				0	C	w.	com.		com.	
Yahaing	Pegu				0	C	w.	com.		com.	
Yahaing-Saw-bywa- Saba	Tavoy				0	C-E	w.	com.		com.	
Yakaw	Henzada				0	C	w.	com.		com.	

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	
7.23	2.30		3.14		c	com.	sl.				
7.02	2.21		3.18		c-e	com.	0	1.43	2.072	1.4489	Glumes lighter colored at base.
7.13	2.30	1.86	3.10	1.23	c	com.	0	1.50	2.116	1.4107	763
7.07	2.25		3.14		c	com.	sl.	1.43	2.120	1.4825	
7.50	2.22		3.38		e	com.	0	1.51	2.134	1.4132	
6.96	2.31		3.01		e	com.	0				
7.30	2.32	1.85	3.15	1.25	c-e	com.	0	1.54	2.162	1.4039	825
7.05	2.32		3.04		c	com.	sl.	1.57	2.208	1.4063	
7.32	2.32		3.16		c-e	com.	0	1.44	2.138	1.4847	
7.71	2.27		3.40		d	com.	0	1.57	2.210	1.4076	Glumes lighter colored at base.
6.96	2.31	1.74	3.01	1.32	e	com.	0	1.56	2.210	1.4167	812
7.15	2.17	1.89	3.29	1.15	c	com.	sl.		2.328		794
7.45	2.24		3.33		c-d	com.	0	1.65	2.328	1.4109	
6.89	2.28	1.76	3.02	1.29	c	com.	0	1.44	2.014	1.3986	785
7.13	2.34	1.87	3.05	1.25	d-f	com.	sl.	1.70	2.428	1.4282	822
7.32	2.44		3.00		c	com.	sl.				
7.10	2.33		3.05		e	com.	0	1.46	2.114	1.4479	
6.90	2.27		3.04		c	com.	0	1.48	2.100	1.4188	Long-glumed.
7.37	2.29		3.22		c	com.	0	1.59	2.298	1.4452	
7.15	2.24	1.85	3.19	1.21	c-e	com.	sl.	1.36	1.968	1.4471	785
7.13	2.30		3.10		c	com.	0	1.60	2.240	1.4000	
6.85	2.27		3.02		c	b.r.	sl.	1.72	2.472	1.4372	
7.11	2.27		3.13		c	com.	0	1.55	2.166	1.3974	
7.15	2.36		3.03		c	com.	0	1.57	2.236	1.4242	
7.64	2.20		3.48		e	com.	0				
7.04	2.22		3.17		c-e	com.	0				
7.40	2.12		3.49		e	com.	0	1.38	1.934	1.4014	Glumes lighter colored at base.
7.81	2.37		3.29		c	com.	sl.				
7.15	2.34		3.06		c	com.	0	1.56	2.202	1.4680	
7.31	2.41		3.03		c-e	com.	0				
6.97	2.31		3.02		c	com.	sl.	1.49	2.112	1.4174	

Variety	District	Unhulled grain									Specific gravity
		Length			Thickness			Colour of			
		m.m.	Breadth	m.m.	m.m.	Length of awn	m.m.	Empty glumes	Glumes	Awn	Tip of glumes
Et-matät	Myaungmya					0	C	w.	com.	com.	
Kaubyin San IImwe	Pyapon					0	C	w.	com.	com.	
Mywe-pyu Saba	Tavoy					0	E	w.	com.	com.	
Ngasein Saw-Bwa	Pyapon					0	C	w.	com.	com.	
Saw Bwa	Pyapon	9.32	2.66	2.03	0	C	w.	com.	com.	2.796	
Taung-pyan	Tharrawaddy	8.72	2.75	2.07	0	C	w.	com.	com.	2.582	

L L.

N.-G., S., s.

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length m.m.	Proportion between Thickness and Breadth	Shape	Colour	Abdominal white	Volume of c. c. 100 grains	Weight of g. 100 grains	Specific gravity	
6.90	2.23		3.09	c	com.	0		1.41	2.022	1.4340	
6.66	2.22		3.00	c	com.	0		1.49	2.102	1.4107	
6.82	2.21		3.09	c	com.	0		1.57	2.194	1.3974	
6.75	2.25		3.00	c	com.	0		1.55	2.170	1.4000	776
6.80	2.22		3.06	c	com.	sl.		1.992		771	Long glumed.
6.98	2.19	1.77	3.18	1.24	c	com.	0				

**LONG-****L A R**

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of				Specific gravity
							Empty glumes	Glumes	Awn	Tip of glumes	
At-Ma-Hta	Tharrawaddy	9.46	3.08	2.16	0	D	w.	l.o.		com.	3.192
Aung-Bala Myo	Bassein	9.68	3.30	2.26	0-25	E	w.	com.	w.	com.	3.667
Ba-Lu-Gyun	Pyapon				0	B	w.	com.		com.	
Baw-yut	Pyapon	8.25	3.64	2.41	0	A	w.	com.		com.	3.156
Baw-yut Mesut	Myaungmya				0-5	B	w.	com.	l.b.	l.b.	
Bee-loo-gyun	Myaungmya				0	B	w.	com.		com.	
Bee-loo-gyonet	Myaungmya				0	B	w.	com.		com.	
Bilungyun Sangin	Maubin	9.33	3.81	2.45	0	B	w.	com.		com.	4.086
Bodaw	Pyapon				0	A	w.	com.		l.b.	
Bojat	Pyapon				0	F	w.	com.		com.	
Bu-gyi	Myaungmya				0	A	w.	com.		com.	
Byat	Myaungmya				0	A	w.	l.o.		l.o.	
Byat	Thaton				0	F	w.	com.		com.	
Byat-ngakywe	Myaungmya				0	B	w.	drab		drab	
Byat-Saba	Myaungmya	9.15	3.83	2.44	0	B	w.	l.o.		l.o.	1.165
Daik-kouk-kyi Kouk-Hnoun Myo	Bassein				0-24	F	w.	com.	w.	l.b.	
Dalesan I		8.39	3.56	2.40	0	F	w.	com.		com.	3.402
Dalesan II	Myaungmya				0	B	w.	com.		com.	
Dalisan	Myaungmya	8.54	3.69	2.34	0	A	w.	com.		l.b.	3.460
Eit-Ma-Ta	Mergui	9.55	3.03	2.11	0	D	w.	l.o.		l.o.	3.451
Htidawmo- gaung-yaung	Maubin				0	B	w.	com.		com.	
Jedaw-mo					0	B	w.	com.		com.	
Kalagyi	Hemzada				0	B-F	w.	l.o.		l.o.	
Kalagyi	Pegu				0-17	B	w.	l.o.	y.	l.o.	
Kalagyi	Myaungmya				0	A-B	w.	l.o.		l.o.	
Kauk-hmmwe	Thaton	8.84	3.88	2.36	0-10	F	w.	com.	w.	com.	3.924
Kauk-kyi	Myaungmya				0	A-B	w.	com.		com.	

## GRAINED.

G E .

N.-G., L., I.

Hulled grain												Remarks	
Length m.m.	Breadth m.m.	Thickness m.m.	Proportion between		Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	Proportion between wts. of unhulled and hulled grain		
			Breadth and Length	Thickness and Breadth									
7.72	2.62	1.89	2.95	1.39	d	com.	0	1.81	2.512	1.3878	787	Glumes lighter colored at both ends and on veins.	
7.64	2.69	1.98	2.84	1.36	d	com.	med.		2.371		783		
6.50	3.10		2.10		b	com.	med.	2.03	2.780	1.3695		Abd. white Shiratama type.	
6.33	3.15	2.17	2.01	1.45	a	com.	cons.	1.87	2.550	1.3636	808		
6.32	3.10		2.04		b	com.	cons.	1.87	2.560	1.3690			
6.60	3.08		2.14		b	com.	cons.	2.07	2.830	1.3671			
6.90	3.10		2.23		b	com.	cons.	1.95	2.708	1.3887			
7.28	3.28	2.18	2.22	1.50	b	com	cons.	2.34	3.278	1.4009	802		
6.30	3.10		2.03		a	com.	cons.						
6.72	3.13		2.14		f	com.	cons.	1.90	2.648	1.3937			
6.30	3.10		2.03		a	com.	med.	1.90	2.620	1.3789			
7.17	3.30		2.17		a	com.	cons.					Glumes lighter colored on veins.	
7.27	3.15		2.31		f	com.	cons.	2.11	2.902	1.3753			
7.10	3.00		2.37		b	com.	med.	2.26	3.084	1.3646		Abd. white Shiratama type.	
7.26	3.30	2.28	2.20	1.45	b	com.	cons.	2.16	2.976	1.3778		Glumes lighter colored on veins.	
7.12	3.42		2.08		f	com.	cons.						
6.45	2.08	2.13	2.09	1.44	f	com.	sl.		2.745		807	Abd. white Shiratama type.	
6.27	3.13		2.00		b	com.	med.						
6.47	3.11	2.10	2.08	1.48	a	com.	med.		2.809		812	Abd. white Shiratama type.	
7.73	2.66	1.84	2.90	1.45	d	com.	0		2.679		776	Glumes lighter colored on veins.	
6.80	3.20		2.12		b	com.	med.	1.90	2.596	1.3663			
6.55	3.05		2.14		b	com.	med.						
6.85	3.13		2.19		b-f	com.	cons.						
7.00	3.20		2.19		b	com.	cons.					Glumes lighter colored on veins.	
7.16	3.30		2.17		a-b	com.	cons.					Glumes lighter colored on veins.	
7.09	3.36	2.18	2.11	1.62	f	com.	med.	2.26	3.155	1.3960	8.04		
6.40	3.10		2.06		a-b	com.	med.						

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of empty glumes	Glumes	Awn	Tip of glumes	Weight of grains g. 100)
Kauk-kyi Medon	Myaungmya	8.42	3.69	2.40	0	A	w.	com.	l.b.	3.525	
Kauk-kyi Shwewa	Myaungmya				0	A-B	w.	com.	com.		
Kauk-san	Myaungmya				0	B	w.	com.	com.		
Kauk-yin Saba-nan	Myaungmya	9.73	3.03	2.07	0	C-E	w.	l.o.	com.	3.312	
Kaung-nyin-gwado	Myaungmya				0	A-B	w.	com.	com.		
Kawa-kyi	Pyapon				0	A	w.	com.	l.b.		
Kaya-the	Myaungmya				0	B-F	w.	com.	com.		
Kyai-ni-young	Salween	9.18	4.17	2.76	0	F	r.b.	com.	r.b.	4.466	1.165
Letywezin Samala-we	Maubin				0	B	w.	com.	com.		
Longyi	Hemzada				0	B	w.	com.	com.		
Lonpyre	Myaungmya				0	B	w.	com.	com.		
Magyagyi	Pegu				0	B	w.	com.	com.		
Minthagyi	Pyapon	8.34	3.55	2.30	0	B	w.	com.	com.	3.64	
Momaka	Myaungmya				0	B	w.	com.	com.		
Nabihotda	Pyapon				0	C-D	w.	o.	l.o.		
Ngakyauk-medo	Myaungmya				0	B	w.	com.	l.b.		
Nga-kyouk Pekya	Bassein				0	B	w.	com.	com.		
Kauk-Lat-Myo											
Nga-Kyauk-Pyu	Myaungmya				0	B	w.	com.	com.		
Nga-Mayeih	Pyapon				0	A-B	w.	com.	l.b.		
Nga-nyugyi	Sandoway	9.95	3.31	2.29	0	D	w.	com.	com.	3.716	
Nga-Pyu-gyi	Pegu				0	F	w.	com.	com.		
Nga-sein	Myaungmya				0	B	w.	com.	com.		
Nga-sein Balu Saba	Pyapon	8.30	3.51	2.34	0	A	w.	com.	l.b.	3.335	1.210
Nga-sein Byau	Thaton	8.19	3.87	2.41	0	F	w.	com.	l.b.	4.200	1.186
Nga-sein-Gyan	Thaton				0	F	w.	com.	com.		
Nga-sein-mwe	Myaungmya				0	F	w.	com.	b.		
Ngasein-Thee-Dat	Pyapon	8.37	3.61	2.37	0	B	w.	com.	com.	3.348	
Ngwe-ma	Myanngmya				0	B	w.	com.	l.b.		
Nwe-gyi	Myaungmya	8.49	3.60	2.39	0	B	w.	com.	l.b.	3.395	
On-Gaing	Thaton	9.18	3.67	2.43	0-24	B	w.	com.	w.	com.	1.164

Hulled grain											Remarks	
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between Thickness and Breadth	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains	Specific gravity		
6.35	3.14	2.15	2.02	1.46	a	com.	med.		2.885		818	
6.45	3.09		2.09		a-b	com.	med.					
6.40	3.10		2.06		b	com.	cons.					
7.61	2.58	1.82	2.95	1.42	c-e	com.	o		2.602		785	
6.50	3.10		2.00		a-b	com.	med.					
6.30	3.10		2.03		a	com.	med.					
6.66	3.15		2.11		b-f	com.	med.					
7.01	3.49	2.29	2.01	1.52	f	com.	cons.	2.63	3.725	1.4164	834	
6.73	3.03		2.22		b	com.	cons.					
6.60	3.10		2.13		b	com.	cons.					
6.40	3.10		2.06		b	com.	med.					
6.90	3.00		2.30		b	com.	cons.					
6.45	3.13	2.07	2.06	1.51	b	com.	med.		2.789		805	
6.40	3.10		2.06		b	com.	cons.					
7.60	2.70		2.82		c-d	com.	o					
6.40	3.10		2.06		b	com.	med.					
6.30	3.10		2.03		b	com.	cons.					
6.80	2.88		2.36		b	com.	med.					
6.40	3.20		2.00		a-b	com.	med.					
7.80	2.78	1.95	2.81	1.43	d	com.	sl.		3.056		823	
6.90	3.10		2.23		f	com.	cons.					
6.87	3.10		2.22		b	com.	med.					
6.36	3.10	2.10	2.05	1.48	a	com.	med.	1.93	2.730	1.4145	818	
7.10	3.33	2.09	2.13	1.59	f	com.	cons.	2.41	3.371	1.3988	803	
6.60	3.10		2.13		f	com.	cons.					
7.21	2.83		2.55		f	com.	med.					
6.38	3.14	2.03	2.01	1.56	b	com.	med.		2.695		805	
6.40	3.10		2.06		b	com.	med.					
6.53	3.10	2.07	2.10	1.49	b	com.	med.		2.716		800	
6.98	3.17	2.19	2.20	1.44	b	com.	cons.	2.44	3.402	1.3943		

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Tip of glumes	Weight of 100 grains
							Empty glumes	Glumes	Awn		
Pyatgui	Pyapon	8.75	3.83	2.43	0	F	w.	com.		com.	3.864
Saba-gale	Myaungmya				0	B	w.	com.		l.b.	
Saba-phu	Sandoway	9.27	3.32	2.21	0	E-F	w.	com.		l.b.	3.575
Se-Le Kaug Hnyin	Sandoway	9.59	3.60	2.35	0	E	w.	com.		l.b.	3.831
Shit-ya Po	Pegu				0	B	w.	com.		com.	
Shu-ma-nyee	Myaungmya	8.31	3.56	2.41	0	A	w.	com.		l.b.	3.580
Shwechrin	Kyaukpyu				0	A-B	w.	com.		r.b.	
Shwelang-gyi	Myaungmya	8.33	3.62	2.40	0	A	l.b.	com.		l.b.	3.400
Thee-dat	Myaungmya	8.35	3.59	2.33	0	A	w.	com.		l.b.	3.362
Thi-dat	Thaton				0	F	w.	com.		com.	
Thi-Ho Kaub-gyi	Pyapon				0	A	w.	com.		com.	
Zalon Byu	Pyapon	8.55	3.53	2.40	0	B	w.	com.		com.	3.593

## MED

Ai-ma-hta	Myaungmya				0	C	w.	l.o.		com.	
Ashe-Thodoma	Kyaukpyu				0	C-D	w.	com.		com.	
Baite	Akyab				0	C	w.	com.		com.	
Baugauk Kyontama	Maubin				0	C	w.	com.		b.	
Baw-yut	Pegu				0-15	B	w.	com.	w.	com.	
Baw-yut	I	Myaungmya			0-9	B	w.	com.	w.	l.b.	
Baw-yut	II	Myaungmya			0	B	w.	com.		com.	
Baw-yut medon	Myaungmya	8.20	3.19	2.42	0	A	w.	com.		com.	
Baw-yut-meshey	Myaungmya				0-5	A	w.	com.	w.	com.	
Baw-yut-mishe	Hemzada				0-15	A	w.	com.	l.b.	l.b.	
Baw-yut-Kaubgyi	Pyapon				0	A-B	w.	com.		l.b.	
Bec-lat	Myaungmya				0-20	A	w.	l.y.	l.b.	l.b.	
Be-gya	Myaungmya				0	B	w.	com.		com.	
Bekyu	Hemzada				0	B	w.	com.		com.	
Belat-ngasein	Mayuugmya				0	B	w.	com.		com.	

Hulled grain												Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Proportion between Breadth and Length Thickness and Breadth		Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	Proportion between wts. of unhulled and hulled grain	
6.70	3.29	1.93	2.04	1.66	f	com.	med.	2.23	3.075	1.3789	796	
6.30	3.10		2.03		b	com.	med.					
7.45	2.90	1.97	2.57	1.47	e-f	com.	med.		2.971		831	
7.49	2.96	1.93	2.53	1.53	d	com.	med.		3.159		824	
6.60	3.00		2.20		b	com.	med.					
6.50	3.10	2.19	2.09	1.42	a	com.	med.		2.938		821	
6.40	3.10		2.06		a-b	com.	cons.					
6.40	3.13	2.4	2.05	1.46	a	com.	med.		2.666		784	
6.40	3.10	2.11	2.06	1.47	a	com.	med.		2.730		812	
7.30	3.40		2.15		f	com.	cons.					
6.40	3.10		2.06		a	com.	med.					
6.61	2.98	2.12	2.22	1.41	b	com.	med.		2.883		803	

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7.45	2.55		2.92		c	com.	s.l.	1.80	2.510	1.3944		Glumes lighter colored at both ends and on veins.
6.30	2.57		2.45		c-d	com.	o	1.41	1.974	1.4000		
6.79	2.52		2.69		c	com.	s.l.	1.51	2.124	1.4066		
7.45	2.51		2.97		c	com.	o	1.71	2.416	1.4129		
6.40	2.90		2.21		b	com.	med.					
6.34	2.86		2.22		b	com.	cons.	1.83	2.532	1.3836		
6.40	2.83		2.26		b	com.	cons.	1.76	2.468	1.4023		
6.29	3.03	2.11	2.08	1.44	a	com.	cons.	1.83	2.590	1.4153		
6.20	3.00		2.07		a	com.	med.	1.84	2.570	1.3967		Abd. white Shiratama type.
6.05	3.02		2.00		a	com.	med.	1.80	2.484	1.3800		Abd. white Shiratama type.
6.26	3.05		2.05		a-b	com.	med.	1.92	2.684	1.3979		
6.08	3.03		2.01		a	com.	med.	1.87	2.534	1.3818		Abd. white Shiratama type.
6.58	2.96		2.22		b	com.	med.	1.84	2.548	1.3848		Abd. white Shiratama type.
6.04	2.69		2.25		b	com.	med.	1.35	1.922	1.4237		
6.60	2.90		2.28		b	com.	cons.	1.88	2.612	1.3894		

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Tip of glumes	Weight of 100 grains
							Empty glumes	Glumes	Awn		
Bogyi	Toungoo			4	0	A	w.	buff		buff	
Bogyan	Myaungmya				0	B	w.	com.		com.	
Begale	Pegu				0	B	w.	com.		com.	
Bosa	Hemzada				0	F	w.	com.		com.	
But-po	Hemzada				0	C	w.	com.		com.	
Byaug-gyoon	Myaungmya				0	B	w.	com.		com.	
Byat	Pegu				0	B	w.	com.		com.	
Byat-bywa	Tharrawaddy	7.72	3.43	2.20	0	A	w.	l.y.		l.y.	2.777
Byat-kate	Myaungmya	7.70	3.13	2.23	0	B	w.	com.		com.	2.547
Byat-saba	Kyaukpyu				0	A-B	w.	com.		l.b.	
Byat-saba	Myaungmya				0	B	w.	com.		com.	
Chartha-gyi	Myaungmya				0	B	w.	com.		com.	
Da-le-san	Ssndoway	8.57	3.40	2.35	0	B	w.	com.		dar.b.	3.238
Deik	Hemzada				0	B	w.	com.		com.	
Dume	Pegu				0	A	w.	com.		l.b.	
Gowmakon	Kyaukpyu				0	C	w.	com.		com.	
Graylaung	Kyaukpyu	8.81	3.01	2.13	0-20	C	w.	com.	w.	com.	2.750
Graungui	Hemzada				0	B	l.b.	com.		b	
Gwadæ Myathla chaung.	Maubin				0	A	w.	l.y.		l.y.	
Gyikelnie					0	E	dar.b.	com.		dar.b.	
Gyosa-kauk	Myaungmya				0	C	w.	com.		com.	
Hli-Daw-Möh	Sandoway				0	B	w.	com.		com.	
Hmawgum	Hemzada				0	B	w.	com.		com.	
Hmin-gale	Myaungmya				0	A	w.	com.		com.	
Hputu	Sandoway				0	C-E	w.	com.		com.	
Hpye-pyugy-saba	Tavoy				0	E	w.	com.		dar.b.	
Hputoo-saba	Tavoy				0-10	A	w.	com.	w.	com.	
Htaw-But Kauk-lat	Sandoway				0	B-C	w.	com.		com.	
Htee-hla	Myaungmya				0	B	w.	com.		com.	
Imata	Hemzada	6.99	3.10	2.08	0	E	w.	l.o.		l.o.	3.233

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	
6.40	2.90		2.21		a	com.	sl.	1.70	2.370	1.3941	
6.53	2.61		2.50		b	com.	med.	1.65	2.252	1.3648	
6.00	2.90		2.07		b	com.	med.				
6.41	2.74		2.34		f	com.	sl.	1.80	2.528	1.4044	Abd. w. Shiratama type.
7.34	2.56		2.87		c	com.	sl.	1.60	2.250	1.4063	
6.80	2.40		2.83		b	com.	med.				
6.10	3.00		2.03		b	com.	med.				
5.73	2.82	1.93	2.03	1.46	a	com.	med.		2.214		797
5.70	2.72	2.00	2.10	1.36	b	com.	med.	1.50	2.081	1.3873	817
5.80	2.60		2.23		a-b	com.	med.	1.58	2.182	1.3810	
6.40	3.00		2.13		b	com.	med.	1.82	2.536	1.3934	
6.24	2.92		2.14		b	com.	cons.				
6.34	2.91	2.09	2.18	1.39	b	com.	cons.	1.86	2.600	1.3978	803
5.75	2.76		2.08		b	com.	cons.	1.57	2.158	1.3745	
6.20	2.80		2.21		a	com.	med.	1.57	2.202	1.4025	
6.12	2.59		2.36		c	com.	sl.	1.39	1.936	1.3928	
6.37	2.62	1.86	2.43	1.41	c	b.r.	sl.	1.52	2.148	1.4131	781
6.00	2.99		2.01		f	com.	cons.				
5.80	2.70		2.15		a	com.	sl.	1.66	2.300	1.3855	
6.57	2.94		2.23		e	com.	sl.	1.83	2.544	1.3902	
6.96	2.38		2.92		c	com.	o				
6.53	2.92		2.24		b	com.	sl.	1.79	2.466	1.3776	
6.14	2.82		2.18		b	com.	med.	1.73	2.420	1.3988	
6.30	2.80		2.25		a	com.	sl.	1.82	2.530	1.3901	
6.65	2.40		2.77		c-e	com.	sl.	1.38	1.878	1.3609	
6.91	2.35		2.94		e	com.	o	1.44	1.998	1.3875	
5.82	2.91		2.00		a	com.	med.				
6.27	2.58		2.43		b-c	com.	sl.	1.50	2.026	1.3507	
6.15	2.95		2.08		b	com.	cons.				
7.55	2.56	1.80	2.95	1.42	e	com.	sl.		2.525		781
											Glumes lighter colored on viens.

Variety	District	Unhulled grain								
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Weight of 100 grains g.
							Empty glumes	Glumes	Awn	
Jedaw-mo	Myaungmya				0	B	w.	com.	com.	
Jumaung-saba	Myaungmya				0	C	w.	com.	com.	
Kaing-soke	Myaungmya				0	B	w.	com.	com.	
Kala-gale	Myaungmya				0	A-B	w.	com.	com.	
Kala-gyi	Myaungmya				0	B	w.	com.	com.	
Kala-saba	Bassein				0	A	w.	l.y.	l.y.	
Kauk-Hmwe	Bassein				0	E	w.	com.	com.	
Kaukgyi Taung-pyu	Sandoway				0	A	l.b.	can- ary	l.b.	
Kauk-Hmwe	Salween				0	E	w.	l.o.	l.o.	
Kauk-hmwe	Toungoo				0	E	w.	l.o.	l.o.	
Kauk-kaw-nyun	Myaungmya				0	D-E	w.	com.	com.	
Kauk-kyi Bawyut	Sandoway				0-10	A	w.	can- ary	r.b.	l.b.
Kauk-kyi-yo-ni	Myaungmya				0	B	w.	com.	com.	
Kauk-kyi-Hpudwai	Sandoway				0	B	w.	com.	com.	
Kauklat Bava	Sandoway				0	B	w.	com.	com.	
Kauk-Lat-Tazindan Lenan Bwa	Sandoway				0	C	w.	com.	com.	
Kauk-ngi Le Manaing	Sandoway				0	B	w.	com.	com.	
Kauk-po	Toungoo				0	A	w.	com.	com.	
Kauk-pyu	Prome				0	C	w.	com.	com.	
Kauk-san	Kyaukpyu				0	C	w.	com.	com.	
Kauk-san I	Myaungmya				0	B	w.	com.	com.	
Kauk-san II	Myaungmya				0	A-B	w.	com.	com.	
Kauk-san	Sandoway				0	B	w.	com.	com.	
Kauk-san-gyi I	Sandoway				0	B	w.	com.	com.	
Kauk-san-gyi II	Sandoway				0	B	w.	com.	com.	
Kauk-san-gyi	Pegu				0	B	w.	com.	com.	
Kauk-sat	Myaungmya				0	B	w.	com.	com.	
Kaukshe	Bassein				0	B	w.	com.	l.b.	
Kauk Thwai Pyu	Pegu				0	C	w.	com.	com.	
Kauk-tungyi	Hemzada				0	B	w.	com.	com.	

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	
6.45	2.73		2.36		b	com.	med.	1.76	2.428	1.3795	
6.55	2.41		2.72		c	com.	o				
6.29	3.10		2.03		b	com.	cons.				
5.93	2.64		2.25		a-b	com.	sl.				
6.60	2.95		2.24		b	com.	med.				
6.12	3.00		2.04		a	com.	med.				
6.80	2.29		2.97		e	com.	o				
6.00	2.90		2.07		a	com.	med.				
7.32	2.52		2.90		d	com.	sl.				
6.87	2.82		2.44		e	com.	o				
6.87	2.42		2.84		d-e	com.	sl.				
6.00	3.00		2.00		a	com.	med.				
6.00	2.90		2.07		b	com.	med.				
6.10	2.90		2.10		b	com.	med.				
6.20	3.00		2.07		b	com.	med.				
6.78	2.36		2.87		c	com.	o				
6.10	2.80		2.18		b	com.	med.	1.74	2.424	1.3931	
6.10	3.00		2.03		a	com.	med.				
6.25	2.52		2.48		c	com.	o				
6.48	2.55		2.54		c	com.	med.				
6.20	2.90		2.14		b	com.	med.				
6.50	2.87		2.26		a-b	com.	med.				
6.15	2.60		2.37		b	com.	med.				
6.00	3.00		2.00		b	com.	cons.				
6.11	2.84		2.15		b	com.	cons.				
6.20	3.00		2.07		b	com.	cons.				
6.68	2.90		2.30		b	com.	med.				
6.15	2.78		2.21		b	com.	med.				
6.75	2.58		2.62		c	com.	sl.				
6.20	2.88		2.15		b	com.	med.	1.80	2.488	1.3822	

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Weight of 100 grains g.	Specific gravity
							Empty glumes	Glumes	Awn		
Kauk-thwe-pyu	Myaungmya	9.81	2.87	2.08	0	C	w.	com.		com.	2.420
Kauktwepyu	Hemzada	8.92	2.70	1.90	0	C	w.	com.		com.	
Kaukwayink	Salween				0	E	w.	com.		com.	2.414
Kauky-a-Saba	Myaungmya				0	B	w.	com.		com.	
Kauk-yin	Myaungmya				0	C-E	w.	com.		l.b.	
Kauk-yin Nga Pyugli	Sandoway				0	C	w.	com.		com.	
Kaw	N. Arakan				0-4	B	w.	com.	w.	com.	
Kaw-kah Nyut	Bassein				0	D-E	w.	com.		com.	
Kawkauyut	Hemzada				0	B	w.	com.		com.	
Kayinchan	Hemzada				0	C	w.	com.		com.	
Kazin	Hemzada				0	C	w.	com.		com.	
Khalu-Thi	Sandoway				0	C	w.	com.		b.	
Khulasan-pyu	Kyaukpyu				0	A	w.	com.		com.	
Khun-lua Kouk-Nge	Bassein				0	C	w.	y.		y.	
Khunwa	Maubin				0	C	w.	y.		y.	
Koe-wai	Myaungmya				0	A	w.	com.		com.	
Kome	Hemzada				0	A	w.	com.		com.	
Kome Magathla-channng	Maubin	Pegu			0	A	w.	com.		com.	
Kyapo	Pegu				0	A	w.	com.		com.	
Kun-gyi	Myaungmya				0	B	w.	com.		com.	
Kun-na-naw	Myaungmya				0	A	w.	l.y.		l.y.	
Kun-nee	Myaungmya				0	B	w.	b.		b.	
Kun-saung	Myaungmya				0	B	w.	com.		com.	
Kunwa	Myaungmya				0	B-D	w.	com.		com.	
Kunwagyи	Toungoo				0	C-E	w.	b.y.		b.y.	
Kwin-lon-Pyu	Myaungmya				0	B	w.	com.		com.	
Kwin-lon-Pyu	Bassein				0	A	w.	com.		com.	
Kwin-lon-gyi	Myaungmya				0	B	w.	com.		com.	
Kwin-wa	Peru				0	C	w.	y.		com.	
Kwin-wa	Myaungmya				0	C	w.	com.		com.	

Hulled grain												Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of 100 grains c. c.	Weight of 100 grains g	Specific gravity	Proportion between wts. of unhulled and hulled grain	
6.85	2.35	1.81	2.91	1.30	c	com.	o	1.40	1.982	1.4157	819	
6.84	2.32	1.81	2.95	1.28	c	com.	o	1.54	2.192	1.4234		
6.96	2.39	1.67	2.91	1.43	d-e	com.	o		2.000		828	
6.44	2.80		2.30		b	com.	sl.	1.58	2.204	1.3949		
6.62	2.42		2.74		c-e	com.	sl.					
6.72	2.41		2.79		c	com.	sl.					
6.00	3.00		2.00		b	com.	med.					
7.23	2.47		2.93		d-e	com.	sl.					
6.21	2.83		2.19		b	com.	med.					
6.82	2.29		2.98		c	com.	sl.					
6.39	2.52		2.54		c	com.	med.					
6.52	2.55		2.56		c	com.	sl.					
5.90	2.90		2.03		a	com.	med.					
6.81	2.51		2.71		c	com.	sl.					
6.01	2.60		2.31		c	com.	sl.					
6.00	2.90		2.07		a	com.	med.					
6.17	2.74		2.25		a	com.	cons.	1.68	2.350	1.3988		
6.00	2.90		2.07		a	com.	med.					
6.30	2.80		2.25		a	com.	med.	1.70	2.386	1.4035		
6.15	2.85		2.16		b	com.	med.					
5.90	2.80		2.11		a	com.	med.					Abd. white Shiratama type.
6.27	2.45		2.56		b	com.	sl.					
5.90	2.90		2.03		b	com.	cons.					
6.59	2.32		2.84		b-d	com.	sl.					
6.69	2.44		2.74		c-e	com.	o					
6.40	2.90		2.21		b	com.	cons.					
6.41	2.82		2.27		a	com.	med.					
6.45	2.50		2.58		b	com.	med.					
6.45	2.65		2.44		c	com.	sl.	1.46	2.024	1.3863		Glumes lighter colored at base & on veins.
6.15	2.29		2.69		c	com.	sl.					

Variety	District	Unhulled grain										Weight of 100 grains	Specific gravity	
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of				Empty glumes	Glumes	Awn	
							Empty glumes	Glumes	Awn	Tip of glumes				
Kyauk yin Mekeye	Sandoway	—	—	—	0	C	w.	com.	com.	com.				
Kyauk yin	Myaungmya	—	—	—	0	B	w.	com.	com.	com.				
Kye-mache	Sandoway	—	—	—	0	C	w.	com.	com.	com.				
Kyettuywe	Bassein	9.51	2.89	2.12	0	C	w.	com.	com.	com.		3.028		
Kyet Seeb	Pyapon	—	—	—	0-5	B	l.b.	com.	l.b.	b.				
Kyi-gan-ma	Myaungmya	—	—	—	0	B	w.	com.	com.	com.				
Kyi-ni-yaung	Myaungmya	—	—	—	0	A	l.b.	l.o.	—	b.				
Kyi-pyu	Hemzada	—	—	—	0-15	B	w.	com.	w.	com.				
Kywe-gyi-sut	Pegu	—	—	—	0	A	w.	com.	com.	com.				
Kywe-mwe	Sandoway	—	—	—	0-15	A	l.b.	l.b.	l.b.	b.				
Kyewewe Kauk-tiyi	Sandoway	—	—	—	5-20	A	l.b.	com.	b.	b.				
Lai-ma-Nine	Sandoway	—	—	—	0	A-B	w.	com.	com.	com.				
Lagale	Myaungmya	—	—	—	0	A-B	w.	com.	com.	com.				
Lan-ba-Sabo	Myaungmya	—	—	—	0	B	w.	com.	com.	com.				
Lauba	Myaungmya	8.69	3.34	2.23	0	B	w.	com.	com.	com.	3.020	1.240		
Lay-manaing	Pyapon	—	—	—	0	B	w.	com.	com.	com.				
Lay-ywe-Zingale	Bassein	—	—	—	0	C	w.	com.	com.	com.				
Lee-Daw-ma	Pegu	—	—	—	0	B	w.	com.	com.	com.				
Le-ma-Naing	Tharrawaddy	—	—	—	0	B	w.	com.	com.	com.				
Le-Ma-Naing	Myaungmya	—	—	—	0	B	w.	com.	com.	com.				
Le-Ma-Naing	Kyaukpyu	—	—	—	0	B	w.	com.	com.	com.				
Lemanaing	Hemzada	—	—	—	0	B	w.	com.	com.	com.				
Lemanaing	Pegu	—	—	—	0	B	w.	com.	com.	com.				
Letkon	Kyaukpyu	—	—	—	0	B	w.	com.	com.	com.				
Let-law-ywe Kauk- Lat }	Sandoway	—	—	—	0	B	w.	com.	com.	com.				
Let-yon	Akyab	8.09	2.77	2.16	0	C	w.	com.	b.	2.619				
Let-yon	Sandoway	—	—	—	0	B	w.	com.	com.	com.				
Let-yon-gyi I	Sandoway	—	—	—	0	A	w.	com.	com.	com.				
Let-yon-gyi II	Sandoway	—	—	—	0	B	w.	com.	com.	com.				
Letyon-Padin-phyu	Akyab	9.38	2.83	2.00	0	C	w.	com.	com.	com.	2.608			

## Hulled grain

Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between		Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	Proportion between wts. of unhulled and hulled grain	Remarks	
				Breadth	Length									
6.90	2.37		2.91		c	com.	sl.							
6.18	2.92		2.12		b	com.	med.							
6.94	2.55		2.72		c	com.	sl.							
7.10	2.45	1.86	2.90	1.32	c	com.	sl.			2.400		793		
5.80	2.80		2.07		b	com.	med.							Abd. white Shiratama type.
6.45	2.80		2.30		b	com.	med.		1.73	2.408	1.3919			
6.28	3.04		2.07		a	com.	cons.							Glumes lighter colored or veins.
6.40	2.90		2.21		b	com.	med.							Abd. white Shiratama type.
6.04	3.02		2.00		a	com.	med.							Abd. white Shiratama type.
5.70	2.85		2.00		a	com.	med.							Abd. white Shiratama type.
6.22	2.83		2.20		a	com.	sl.							Glumes streaked with l. b. color.
5.80	2.90		2.00		a-b	com.	cons.							Abd. white Shiratama type.
6.20	2.90		2.14		a-b	com.	cons.							
6.29	3.00		2.10		b	com.	cons.							
6.40	2.77	2.00	2.31	1.39	b	com.	med.		1.70	2.392	1.4071	792		
5.83	2.82		2.07		b	com.	med.							
6.65	2.55		2.61		c	com.	o							
6.50	2.90		2.24		b	com.	sl.							
6.25	2.82		2.22		b	com.	cons.		1.71	2.378	1.3906			Glumes oft. spotted with b. color.
6.31	2.84		2.22		b	com.	med.		1.67	2.320	1.3892			
5.85	2.84		2.06		b	com.	cons.							Glumes spotted with pur. b. color.
6.10	2.77		2.20		b	com.	med.		1.77	2.430	1.3729			Glumes spotted with b. color.
6.26	2.82		2.22		b	com.	med.		1.79	2.472	1.3810			
5.88	2.78		2.12		b	com.	med.							
6.08	2.64		2.30		b	com.	sl.							
6.22	2.43	1.90	2.56	1.28	c	com.	o			2.012		768		
6.40	2.60		2.46		b	com.	sl.							
5.90	2.90		2.03		a	com.	med.							
6.10	2.85		2.14		b	com.	sl.							
7.09	2.39	1.71	2.97	1.40	c	com.	o			2.040		782		

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Weight of 100 grains	Specific gravity
							Empty glumes	Glumes	Awn		
Letyweegin-longgegi Dawebyu }	Maubin			6	0-20	B	w.	com.	w.	com.	
Letyway-zin	Pegu				0	B	w.	com.		com.	
Letywesin I	Tharrawaddy				0	B	w.	com.		com.	
Letywe-sin II	Tharrawaddy				0	B	w.	com.		com.	
Letywesin	Myaungmya				0	B	w.	com.		com.	
Letywezin I	Myaungmya				0	B	w.	com.		com.	
Letywezin II	Myaungmya				0	B	w.	com.		com.	
Letywezin	Pyapon				0	B	w.	com.		com.	
Letywezin	Toungoo	9.00	3.10	2.23	0	B	w.	com.		com.	2.984
Letywezingal	Hemzada				0	B	w.	com.		com.	
Letywezingyi Kauk-Lat-Myo }	Bassein				0	B	w.	com.		com.	
Letywezin Kauk-Lat-Myo }	Bassein				0	B	w.	com.		com.	
Lon-Bu	Pegu				0	B	l.b.	com.		l.b.	
Lon-Byu	Sandoway				0	B	w.	com.		com.	
Londat	Bassein				0	B	w.	com.		com.	
Longyi Saba	Tavoy	8.87	2.95	2.18	0	C	l.b.	com.		b.	2.900
Lonpu	Hemzada				0	A	w.	l.y.		l.y.	
Lonpyu	Kyaukpyu				0	B	w.	com.		com.	
Lonthwe	Myaungmya				0	C	w.	com.		com.	
Lu	Myaungmya				0	B	w.	com.		com.	
Ma-Ay-Ngasein	Bassein				0	B	w.	com.		com.	
Madawa-Saba	Myaungmya				0-8	B	l.b.	com.	b.	b.	
Mai-Nu Kauk-Le- Myo }	Bassein				0	B	w.	com.		com.	
Mayin-saba	Bassein				0	C	w.	com.		com.	
Mayanwe	Sandoway	8.58	2.95	2.19	0	B	w.	com.		dar. b.	2.863
Medan-Thwe	Pyapon				0	A-B	w.	com.		l.b.	
Meiu-skahla	Hemzada				0	C	w.	com.		com.	
Meso	Pyapon				0-10	A	w.	l.y.	w.	l.b.	
Meta	Hemzada				0	B	w.	com.		com.	
Mga-sein Kywe	Myaungmya				0	B	w.	com.		com.	

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	
6.23	2.74		2.27		b	com.	med.				
6.24	2.52		2.48		b	com.	sl.				
6.57	2.67		2.46		b	com.	sl.				
6.33	2.68		2.36		a	com.	sl.				
6.37	2.83		2.25		b	com.	sl.				
6.38	2.64		2.42		b	com.	sl.				
6.65	2.72		2.44		b	com.	med.				
6.58	2.65		2.48		b	com.	med.				
6.16	2.65	1.90	2.32	1.39	b	com.	sl.	2.333		783	
6.38	2.68		2.38		b	com.	med.				
6.32	2.92		2.16		b	com.	sl.				
6.56	2.85		2.30		b	com.	sl.				
5.90	2.90		2.03		b	com.	med.				
5.80	2.80		2.07		b	com.	med.				
5.80	2.90		2.00		b	com.	med.				
6.85	2.61	1.90	2.62	1.37	c	com.	sl.	2.406		830	
5.80	2.88		2.01		a	com.	med.				Abd. white Shiratama type.
6.10	2.60		2.35		b	com.	sl.				
6.40	2.38		2.69		c	com.	o				
6.40	2.60		2.46		b	com.	med.				
6.55	2.44		2.68		b	com.	sl.				
5.60	2.80		2.00		b	com.	sl.				Abd. white Shiratama type.
5.90	2.95		2.00		b	com.	cons.				
6.92	2.44		2.84		c	com.	sl.				
6.66	2.49	1.92	2.67	1.30	b	com.	sl.	2.254		787	Glumes oft. spotted with purp. b. color.
6.30	2.90		2.17		a-b	com.	med.				Abd. white Shiratama type.
7.08	2.48		2.85		c	com.	sl.				Abd. white Shiratama type.
6.10	3.00		2.03		a	com.	med.				Abd. white Shiratama type.
6.45	2.95		2.19		b	com.	med.				
6.18	2.63		2.35		b	com.	sl.				

Variety	District	Unhulled grain										Specific gravity	
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of						
							Empty glumes	Glumes	Awn	Tip of glumes			
Midan-The	Pyapon			0	A-B	w.	com.		com.				
Minthagyi	Hemzada			0	A	w.	com.		com.				
Moat-Seek-Yin	Sandoway			0-15	B	w.	com.	w.	l.b.				
Moke-Seik I	Myaungmya			0	B	w.	com.		com.				
Moke-Seik II	Myaungmya			0-20	B	w.	com.	w.	l.b.				
Moke-Seikgyi	Myaungmya			0-15	B	w.	com.	w.	l.b.				
Moke-Seik-Kale	Myaungmya			0-10	B	w.	com.	w.	l.b.				
Moke-Seik-Kyi I	Myaungmya			0-10	B	w.	l.y.	b.	b.				
Moke-Seik-Kyi II	Myaungmya			0-6	B	w.	com.	l.b.	com.				
Moke-Seik-kyi	Hemzada			0-10	B	w.	com.	w.	l.b.				
Moke-Seik-Kyi-Saba	Pyapon			0-15	B	w.	com.	w.	com.				
Moke-Seik-Midon	Myaungmya			0-5	B	w.	com.	w.	l.b.				
Myauk-san	Hemzada			0	B	w.	com.		com.				
Mya-yan-baung	Myaungmya			0	B	w.	l.y.		l.y.				
Myossan	Sandoway			0	A-B	w.	com.		com.				
Nat-kun-in	Hemzada			0	A	w.	com.		com.				
Nat Kun	Sandoway			0	B	w.	com.		com.				
Nat Saung Kauk Lat	Sandoway			0	C	w.	com.		com.				
Nautwe	Myaungmya			0	B	w.	com.		com.				
Ngakyauk-Ke	Sandoway	8.11	3.47	2.22	0	A	w.	com.	com.	2.819			
Nga-Hlagyaw Yodaya	Sandoway				0	C	w.	com.	l.b.				
Ngah-ya-gyaw	Thaton				0	B	w.	com.	com.				
Ngakyauk-Kale	Bassein				0	B	w.	com.	com.				
Ngakyauk-Kale	Myaungmya				0	B	w.	com.	com.				
Ngakyauk I	Myaungmya				0	B	w.	com.	com.				
Ngakyauk II	Myaungmya				0	B	w.	com.	l.b.				
Ngukyauk III	Myaungmya				0	B	w.	com.	com.				
Ngakyauk IV	Myaungmya				0	B	w.	com.	com.				
Ngakyauk Begya	Myaungmya				0	B	w.	com.	l.b.				
Ngakyaukbekya	Hemzada				0	B	w.	com.	com.				

Hulled grain										Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains	Specific gravity	
6.20	3.10		2.00	a-b	com.	med.				
6.35	2.80		2.27	a	com.	med.				
6.20	2.90		2.14	b	com.	cons.				
6.02	2.75		2.19	b	com.	med.				
6.50	2.83		2.30	b	com.	med.				
6.30	2.74		2.30	b	com.	med.				
6.67	2.70		2.47	b	com.	med.				
5.50	2.75		2.00	b	com.	med.				Abd. w. Shiratama type.
6.62	2.80		2.36	b	com.	med.				
6.09	2.85		2.14	b	com.	med.				
6.41	2.78		2.31	b	com.	med.				
6.43	2.82		2.28	b	com.	med.				
6.07	2.68		2.26	a	com.	med.				
6.40	2.70		2.37	b	com.	med.				
5.78	2.86		2.02	a	com.	med.				
6.13	2.79		2.20	a	com.	med.				
5.85	2.80		2.09	b	com.	med.				
6.71	2.32		2.89	c	com.	o				
6.20	2.90		2.14	b	com.	med.				
5.75	2.84	2.01	2.02	1.41	a	com.	med.	2.256	800	
6.40	2.70		2.37	c	com.	sl.				
6.05	3.00		2.02	b	com.	cons.				
5.67	2.65		2.14	b	com.	med.				
6.13	2.61		2.35	b	com.	sl.				
5.95	2.61		2.28	b	com.	med.				
6.46	2.66		2.43	b	com.	sl.				
6.10	2.90		2.10	b	com.	med.				
6.40	3.00		2.13	b	com.	cons.				
6.07	2.75		2.21	b	com.	cons.				
6.20	2.96		2.09	b	com.	cons.				Glumes spotted with b. color.

Variety	District	Unhulled grain								
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			
							Empty glumes	Glumes	Awn	Tip of glumes
Ngakyauk-kayan	Myaungmya				0	B	w.	com.		com.
Ngakyauk	Tharrawaddy				0	B	w.	com.		com.
Ngakyauk	Toungoo				0	B	w.	com.		com.
Ngakyauk-kyi	Hemzada				0	B	w.	com.		l.b.
Ngakyauk-kyi	Pegu	8.59	3.21	2.26	0	B	w.	com.	com.	3.098 1.239
Ngakyauk-me	Myaungmya				0	B	w.	com.		l.b.
Nga-kyauk-Mwe	Myaungmya				0	B	w.	com.		l.b.
Nga-kyauk-Ni	Pegu				0	B	w.	com.		com.
Ngakyauk Pyu	Tharrawaddy				0	B	w.	com.		com.
Ngakyauk Nu-twa	Myaungmya				0	B	w.	com.		com.
Ngakyauk Pyu	Bassein				0	B	w.	com.		l.b.
Ngakyauk Pyu-gyi	Myaungmya				0	B	w.	com.		com.
Ngakyauk pyu-saba	Pyapon				0	B	w.	com.		com.
Ngakyauk-yin	Myaungmya				0	B	w.	com.		com.
Ngakyi-Ma	Pegu				0	B	w.	com.		com.
Nga-kyi-Masalay	Pegu				0	B	w.	com.		com.
Ngakyauk Ke	Kyaukpyu				0	B	w.	com.		com.
Nga-kyouk	Pyapon				0	B	w.	com.		com.
Nga-Kyouk Pyu Kauk-Nge	Bassein				0	B	w.	com.		com.
Nga-Kyouk-yin Kouk-Yin-Myo	Bassein				0	B	w.	com.		com.
Nga-kywe	Myaungmya				0	A	w.	drab		dar. drab
Nga-kywe Byu	Pyapon				0-10	B	w.	com.	w.	com.
Ngakwe Pyu	Hemzada				0-15	B	w.	com.	w.	com.
Nga-Moe-Kaing	Kyaukpyu				0	B	w.	com.		com.
Nga-Mo-yeik	Myaungmya				0	A-B	w.	com.		com.
Nga-moysik Ngasein	Pyapon				0	A	w.	com.		com.
Nga-Mwe	Akyab				0-8	B	w.	com.	w.	com.
Nga-Mwe	Sandoway	8.90	3.02	2.09	0	B	w.	com.		com. 2.792
Nga-Mwe Saba	Kyaukpyu				0-5	B	w.	com.	w.	com.
Ngamyaucksam	Hemzada				0	B	w.	com.		com.

Hulled grain												Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Proportion between Breadth and Length Thickness and Breadth		Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g	Specific gravity	Proportion between wts. of unhulled and hulled grain	
6.02	2.70		2.23		b	b.r.	sl.					
6.17	2.85		2.16		a	com.	cons.					
6.30	2.78		2.27		b	com.	sl.					
6.37	2.74		2.32		b	com.	sl.					
5.93	2.58	1.99	2.30	1.31	b	b.r.	sl.	1.73	2.435	1.4075	786	
6.36	2.57		2.47		b	com.	med.					
6.58	2.72		2.42		b	com.	sl.					
6.36	2.60		2.45		b	b.r.	med.					
6.30	3.00		2.10		b	com.	cons.					
6.30	2.90		2.17		b	com.	med.					
6.47	2.71		2.39		b	com.	sl.					
6.10	2.70		2.26		b	com.	med.					
5.83	2.63		2.22		b	com.	med.					
6.37	2.91		2.19		b	com.	med.					
6.04	2.76		2.19		b	com.	med.					
6.33	2.57		2.46		b	com.	med.					
5.90	2.90		2.03		b	com.	med.					
6.20	2.75		2.25		b	com.	med.					
6.62	2.77		2.39		a	com.	cons.					
6.50	2.90		2.24		b	com.	med.					
6.00	2.90		2.07		a	com.	cons.					Abd. white Shiratama type.
6.40	2.96		2.16		b	com.	med.					
6.40	2.84		2.25		b	com.	cons.					
6.00	2.68		2.24		b	com.	cons.					
6.20	3.10		2.00		a-b	com.	med.					
6.05	2.91		2.08		a	com.	cons.					Abd. white Shiratama type.
6.59	2.55		2.66		b	com.	sl.					
6.75	2.56	1.81	2.64	1.41	b	com.	sl.		2.198			787
6.30	2.45		2.57		b	com.	med.					
6.10	2.50		2.44		b	com.	med.					



Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Proportion between Breadth and Length Thickness and Breadth		Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	
6.70	2.49		2.69		b	com.	sl.				
6.40	3.00		2.13		b	com.	cons.				
6.36	2.73		2.33		b	com.	sl.				
6.08	2.74		2.22		b	com.	sl.				
6.30	3.02	2.04	2.09	1.48	a	com.	med.	1.82	2.578	1.4165	803 Abd. white Shiratama type.
6.10	2.90		2.10		b	com.	med.				
6.00	2.90		2.07		b	com.	med.				
6.30	2.70		2.33		b	com.	med.				
6.20	3.00		2.07		a	com.	med.				
6.00	2.87		2.09		a	com.	med.				
6.50	2.90		2.24		b	com.	med.				
6.47	2.88		2.25		b	com.	med.				
6.00	2.87		2.09		b	com.	med.				
6.05	2.70		2.24		b	com.	med.				
6.25	2.63		2.38		b	com.	sl.				
6.30	2.90		2.17	a-b	com.	med.					Abd. white Shiratama type.
6.05	2.74		2.21		b	com.	med.				
6.40	2.80		2.29		b	com.	med.				
6.22	2.77		2.25		b	com.	med.				
6.15	3.00		2.05		b	com.	med.				
6.00	2.86		2.10		a	com.	med.				
6.12	2.78		2.20		b	com.	med.				
6.90	2.55		2.71		b	com.	med.				
6.30	2.80		2.25		b	com.	med.				
6.05	2.83		2.14		b	com.	sl.				
5.75	2.82		2.04		a	com.	med.				Abd. white Shiratama type.
6.40	2.90		2.21	a-b	com.	med.					
6.00	3.00		2.00		a	com.	med.				
6.60	2.70	1.91	2.44	1.44	b	b.r.	med.	1.61	2.300	1.4285	793
5.98	2.62	1.91	2.28	1.37	b	com.	sl.	1.47	2.089	1.4211	792

Variety	District	Unhulled grain								
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Weight of 100 grains g.
							Empty glumes	Glumes	Awn	
Nagsein Ni	Thaton				0	B	w.	com.		l.b.
Ngasein Nyaung doon }	Pyapon				0	B	w.	com.		com.
Ngasein Pin-me I	Myaungmya				0	B	w.	com.		l.b.
Ngasein Pin-me II	Myaungmya				0	B	w.	com.		com.
Ngasein Pin-mee	Myaungmya				0	B	w.	com.		l.b.
Ngasein Pyu	Myaungmya				0	B	w.	com.		l.b.
Ngasein Thee-dat	Myaungmya				0	B	w.	com.		com.
Ngasein Thme-Nwe	Pyapon				0	F	w.	com.		com.
Ngasein yo-nee	Myaungmya				0	B	w.	com.		com.
Ngasein yoni	Myaungmya				0	B	w.	com.		b.l.
Nga-shint Thweay	Salween				0-6	E	r.b.	r.b.	r.b.	b.
Ngat Kyilet-the	Akyab				0	C	w.	com.		com.
Nga-tyu-gyi	Kyaukpyu				0	C	w.	com.		com.
Nga-wo-yeik	Myaungmya				0	B	w.	com.		com.
Nga-ya-ba	Myaungmya				0	B	w.	com.		com.
Nga-ya-bo	Sandoway				0	E	w.	com.		com.
Nga-ya-bo I	Myaungmya				0	B	w.	com.		com.
Nga-ya-bo II	Myanngmya				0	B	w.	com.		com.
Nga-ya-bo III	Myaungmya				0	B	w.	com.		com.
Ngayabo	Bassein				0	B	w.	com.		com.
Nga-ya-po	Pyapon				0	B	w.	com.		com.
Nga-yoon	Tharrawaddy				0	B	w.	com.		com.
Nyu-dan-yin	Sandoway				0	B	w.	com.		com.
On-sa-pa	Tharrawaddy				0	B	w.	com.		com.
Pa-don	Mergui									
Pale-byan	Myaungmya				0-20	A	l.b.	com.	l.b.	b.
Pan-bila	Kyaukpyu				0	B	w.	o.		o.
Pat-lee-gyi	Salween				0	C	w.	com.		dar.b.
Paw-daw-mu	Myaungmya				0	B	w.	com.		com.
Pe-gu-ngakyauk	Myaungmya				0	B	w.	com.		com.

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains	Specific gravity	
6.40	2.85		2.25		a	b.r.	cons.				
6.66	2.90		2.30		b	com.	med.				
6.30	2.75		2.29		b	com.	med.				
6.30	2.70		2.33		b	com.	med.				
6.43	2.92		2.20		b	com.	med.				
5.95	2.77		2.15		b	com.	sl.				
5.90	2.80		2.11		b	com.	med.				
6.62	2.75		2.41		f	com.	med.				
6.20	3.00		2.07		b	com.	cons.				
6.22	2.75		2.26		b	com.	med.				
6.42	2.78		2.31		e	com.	med.				
7.04	2.43		2.90		c	com.	0				
7.03	2.41		2.92		c	com.	sl.				
6.55	2.91		2.25		b	com.	med.				
6.00	2.90		2.07		b	com.	med.				
6.73	2.61		2.58		d	com.	sl.				
6.00	2.90		2.07		b	com.	med.				
6.12	2.72		2.25		b	com.	med.				
6.40	2.80		2.29		b	com.	cons.				
5.90	2.70		2.19		b	com.	sl.				
5.80	2.80		2.07		b	com.	med.				
6.10	2.90		2.10		b	b.r.	med.				
6.22	2.77		2.25		b	com.	med.				
6.17	2.85		2.16		b	com.	med.				
5.72	2.70		2.12								
5.90	2.80		2.11		a	com.	med.				
6.34	2.70		2.35		c	com.	sl.				
7.52	2.53		2.97		c	com.	0				
6.74	2.78		2.42		b	com.	med.				
6.33	2.74		2.31		b	com.	cons.				

Abd. white Shiratama type.

Abd. white Shiratama type.

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of				Weight of 100 grains
							Empty glumes	Glumes	Awn	Tip of glumes	
Phwehtaung Pyu Saba	Tavoy			4	0	B	w.	com.		com	
Pin-yin-ni	Sandoway				0	B	w.	com.		b.	
Pin-yin-Ni	Kyaukpyu				0	C	w.	l.o.		l.o.	
Potun	Hemzada				0	B	w.	com.		com.	
Pyo-lagkin	Mergui				0	B	w.	com.		com.	
Pyu-dow	Myaungmya				0	B	w.	com.		com.	
Pyu-gale I	Myaungmya				0	B	w.	com.		l.b.	
Pyu-gale II	Myaungmya				0	B	w.	com.		com.	
Pyu-gale III	Myaungmya				0	B	w.	com.		com.	
Pyu-mee	Pegu				0	D	w.	com.		com.	
Pyulet-yon	Sandoway				0	B	w.	com.		com.	
Ratsun	N. Arakan				0	E	w.	com.		com.	
Rin-bi	Kyaukpyu				0	B	w.	com.		b.	
Saawhme	Kyaukpyu				0	B	w.	com.		l.b.	
Saba-ib	Pyapon				0	B	w.	b.		b.	
Saba-chamg	Sandoway				0	B	w.	com.		com.	
Sabagale	Kyaukpyu				0	B	w.	com.		com.	
Suba-nee	Myaungmya				0	C	w.	l.o.		l.o.	
Saba-nee	Sandoway				0	B	w.	l.o.		l.o.	
Saba-ni	Myaungmya				0	C-D	w.	l.o.		l.o.	
Sabani Kauk-lat-myoo	Bassein				0	E	w.	l.o.		l.o.	
	Sandoway				0	A	w.	com.		com.	
Saba-pyu-gale	Bassein				0	C	w.	com.		com.	
Saba-pyu-yin	Myaungmya				0	A	w.	com.		com.	
Saba-wa	Myaungmya				0	B	w.	l.o.		l.o.	
Sabaya Kauk-lat-myoo	Bassein				0	A-C	w.	com.		b.	
	Thaton				0	B	w.	com.		com.	
Sangin Momakha	Maubin				0	B	w.	com.		com.	
Sanni-gewgyaung	Maubin	9.14	3.18	2.20	0-5	F	w.	com.	w.	com.	3.014
Sanni-gyi	Sandoway				0	B	w.	com.		com.	1.180

## Hulled grain

Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between Thickness and Breadth	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	Proportion between wts. of unhulled and hulled grain	Remarks
6.18	2.52		2.45		b	com.	sl.					
6.20	3.00		2.07		b	com.	med.					
6.28	2.50		2.51		c	com.	sl.					
5.92	2.86		2.07		b	com.	cons.					
5.80	2.60		2.23		b	com.	med.					
6.00	2.80		2.14		b	com.	med.					
6.11	2.72		2.25		b	com.	med.					
6.47	2.72		2.38		b	com.	sl.					
6.20	2.70		2.30		b	com.	med.					
6.78	2.43		2.79		d	com.	0					
6.40	2.90		2.21		b	com.	med.					
6.51	2.44		2.67		e	com.	0					
5.90	2.64		2.23		b	b.r.	cons.					
6.21	2.45		2.53		b	com.	med.					
6.48	2.35		2.76		b	com.	sl.					
5.90	2.80		2.11		b	com.	cons.					
6.36	2.49		2.55		b	com.	0					
6.67	2.35		2.84		c	com.	sl.					
6.50	2.43		2.67		b	com.	med.					
6.55	2.45		2.67	c-d	com.	sl.						
6.63	2.48		2.67		e	com.	med.					
6.10	2.83		2.16		a	com.	med.					
6.45	2.49		2.59		c	com.	sl.					
6.18	2.82		2.19		a	com.	med.					
6.60	2.40		2.75		b	com.	sl.					
6.69	2.55		2.62	a-c	com.	sl.						
6.40	2.69		2.38		b	com.	med.					
6.42	2.98		2.15		b	com.	med.					
6.72	2.69	1.90	2.50	1.41	f	b.r.	sl.	1.73	2.407	1.3913	799	
6.43	2.63		2.44		b	b.r.	0					

Variety	District	Unhulled grain									Specific gravity	
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Tip of glumes		
							Empty glumes	Glumes	Awn			
Saohme	Myaungmya			1	0-17	B	w.	com.	w.	b.		
Sapaneb-meshe	Pegu				0-17	B	w.	drab	b.	drab		
Sapapyu	Hemzada				0	A	w.	com.		com.		
Sapazat	Hemzada				0	B	r.b.	com.		r.b.		
Sat-me	Myaungmya				0	B	w.	com.		b.		
Sat-mee	Myaungmya				0	B	w.	com.		com.		
Sawgan	Sandoway											
Sabapyu Kaukyin	Tharrawaddy				0	B	w.	com.		com.		
Sein-Daing-gyi	Pegu				0	B	w.	com.		com.		
Sein-gyi-Saba	Tavoy				0-5	A-B	w.	com.	w.	com.		
Sein-ta-ket-mya	Pegu				0	B	w.	com.		com.		
Set-pyan	Myaungmya				0	A	w.	com.		l.b.		
Set-sagaung-Pyu	Henzada				0	B	w.	com.		com.		
Shithnan	Hemzada				0	B	w.	com.		com.		
Shwelaungyi	Myaungmya	8.17	3.49	2.40	0	A	w.	com.		l.b.	1.200	
Shwe-thwe	Mergui				0-6	B	w.	com.	w.	com.		
Shwe-thwe Saba	Tavoy				0	B	w.	com.		com.		
Shwe-wa-cale	Pegu				0	E	w.	y.		y.		
Sin-chi	Sandoway	8.23	3.09	2.10	0-3	B	w.	com.	w.	com.	2.520	
Sit-sa-gaung	Tharrawaddy	8.43	2.76	2.09	0	B	w.	l.o.		l.o.	1.211	
Ta-daung-bo	Myaungmya				0	A	w.	com.		com.		
Ta-daung Ko	Pegu				0	B	w.	com.		com.		
Tadaung Bo	Pegu				0	B	w.	com.		com.		
Taing Byan	Sandoway				0	B	w.	com.		com.		
Tako-ta-loke	Myaungmya				0	B	w.	com.		com.		
Tataung Bo	Bassein				0	B	w.	com.		com.		
Tataung Po-kauk-kyi	Bassein				0	A	w.	com.		com.		
Taung-bay	Tharrawaddy				0-4	B	w.	com.	1b.	b.		
Taung-Kyaw	Prome				0	E	w.	com.		com.		
Taung Pan Byu	Pegu				0	A	w.	com.		com.		

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity		
							Proportion between wts. of unhulled and hulled grain				
6.36	2.78	2.20		b	b.r.	med.					
6.00	2.90	2.07		b	com.	med.					Abd. white Shiratama type.
6.49	2.64	2.46		b	com.	med.					
5.60	2.80	2.00		b	com.	med.					
6.35	2.63	2.41		b	com.	med.					
6.28	2.64	2.38		b	com.	sl.					
5.90	2.70	2.19									
6.27	2.52	2.49		b	com.	sl.					
6.00	2.60	2.31		b	com.	med.					
5.92	2.89	2.05		a-b	com.	cons.					
6.30	2.80	2.25		b	com.	cons.					
6.00	3.00	2.00		a	com.	med.					Abd. white Shiratama type.
6.40	2.48	2.58		b	com.	med.					
6.30	2.84	2.22		b	com.	cons.					
6.29	2.99	2.09	2.10	a	com.	med.	1.92	2.723	1.4182		Abd. white Shiratama type.
6.59	2.55	2.58		b	com.	0					
6.32	2.51	2.52		b	com.	sl.					
6.35	2.38	2.67		e	com.	sl.					
5.96	2.57	1.89	2.32	b	b.r.	cons.	1.44	2.012	1.3972	798	
6.37	2.36	1.83	2.70	1.29	b	com.	sl.	1.939		776	
6.12	2.85	2.50		a	com.	cons.					
6.30	2.70	2.33		b	com.	med.					
6.04	2.68	2.58		b	com.	sl.					Long glumed.
6.00	3.00	2.00		b	com.	cons.					
6.15	2.88	2.14		b	com.	cons.					
6.24	2.74	2.28		b	com.	sl.					
6.20	2.93	2.12		a	b.r.	cons.					
6.57	2.68	2.45		b	com.	med.					Glumes spotted with b. color.
7.21	2.45	2.94		e	com.	sl.					
6.10	3.00	2.03		a	com.	med.					Long glumed. Abd. white Shiratama type.

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Tip of awn	Weight of 100 grains
							Empty glumes	Glumes	Awn		
Taung Paw Yahaing	Bassein				0	B	w.	buff		com.	
Taung-yo-pyu	Tharrawaddy				0	B	w.	o.		o.	
Taw-bat	Myaungmya				0	C	w.	com.		com.	
Tha Dun Byu	Pegu				0	B	w.	com.		com.	
Thaye	Henzada				0-25	B	w.	com.	w.	com.	
Thazindan	Kyaukpyu				0	B	w.	com.		com.	
Thee-dat I	Myaungmya				0	B	w.	com.		com.	
Thee-dat II	Myaungmya				0	B	w.	com.		com.	
Theedat Kauk-kyi	Myaungmya				0	A	w.	com.		com.	
Theedut	Pegu				0	A-B	w.	com.		com.	
Thee-gyi-Saba	Myaungmya				0	B	w.	com.		com.	
Thetat Kaunge	Henzada				0	D	w.	com.		com.	
Thidat I	Bassein				0	B	w.	com.		com.	
Thidat II	Bassein										
Thi-hla Kauk-lat- myo	Bassein				0	B	w.	com.		com.	
Thonla	Akyab	8.20	3.34	2.28	0	B	w.	com.		com.	2.753
Thon-Lon Pyut	Pegu				0	B	w.	com.		com.	
Thu-tay-gyi	Myaungmya				0	B	w.	com.		com.	
Tidaw-mo	Pyapon	8.70	3.36	2.20	0	B	w.	com.		com.	3.204
Tin-aw	Myaungmya				0	D	w.	com.		com.	
Tin-aw	Bassein	8.90	2.91	2.10	0	D-E	w.	com.		com.	2.786
Tin-aw Saba	Myaungmya				0	D	w.	com.		com.	
Titaw-mo	Tharrawaddy				0	B	w.	com.		com.	
Tok-tok-ma	Tharrawaddy				0	B	w.	com.		com.	
Tok-tokena	Prome				0	B	w.	com.		com.	
Topyu	Bassein				0	A	w.	com.		com.	
Toungoo	Myaungmya				0	A-F	w.	com.		com.	
Tou-kaw	Prome				0	D	w.	com.		com.	
Tsan Byant	Salween	9.64	2.98	2.09	0	D	w.	l.o.	l.o.	com.	3.041
Unbank	N. Arakan				0	D	w.	com.		com.	

Hulled grain												Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Proportion between Breadth and Length Thickness and Breadth		Shape	Colour	Abdominal white	Volume of 100 grains c. c.	Weight of 100 grains g.	Specific gravity	Proportion between wts. of unhulled and hulled grain	
6.72	2.67		2.52		b	com.	cons.					Glumes lighter colored toward base.
6.44	2.56		2.52		b	com.	sl.					
6.88	2.32		2.97		c	com.	0	1.60	2.282	1.4263		
6.10	2.90		2.10		b	com.	cons.					
6.20	3.00		2.07		b	com.	cons.					
6.21	2.58		2.41		b	com.	sl.					
6.40	2.70		2.37		b	com.	med.					
6.00	2.86		2.10		b	com.	cons.					
6.47	3.01		2.15		a	com.	med.					Abd. white Shiratama type.
6.19	2.86		2.16		a-b	com.	med.					
6.01	2.75		2.19		b	com.	med.					
7.02	2.42		2.90		d	com.	med.					
6.61	2.93		2.26		b	com.	med.					
5.70	2.80		2.04									
6.20	2.70		2.30		b	com.	cons.					
5.87	2.74	1.98	2.14	1.38	b	com.	med.		2.216		805	
6.10	2.80		2.18		b	com.	cons.					
6.10	3.00		2.03		b	com.	cons.					
6.30	2.90	1.95	2.17	1.49	b	com.	med.	1.83	2.575	1.4071	804	
7.08	2.58		2.74		d	com.	med.					
6.78	2.45	1.70	2.77	1.44	d	com.	sl.		2.126		763	
6.84	2.48		2.76		d	com.	sl.					
6.50	2.75		2.36		b	com.	cons.					
6.31	2.52		2.50		b	com.	med.					
6.60	2.67		2.47		b	com.	med.					
6.09	2.68		2.27		a	com.	med.					
6.19	2.58		2.40		a-f	com.	med.					Glumes have a pinkish tint.
6.65	2.63		2.53		d	com.	med.					
7.29	2.50	1.79	2.92	1.40	d	com.	sl.		2.441		803	Glumes lighter colored on veins.
6.92	2.44		2.84		d	pur. bl.	0					

Variety	District	Unhulled grain										Weight of 100 grains g.	Specific gravity		
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Awn	Tip of glumes				
							Empty glumes	Glumes	Glumes						
Ya-haing	Bassein				0	B	w.	o.	o.						
Yahan	Sandoway				0	D-F	w.	o.	o.						
Ye-na-naing	Myaungmya				0	A-B	w.	com.	l.b.						
Yodaya I	Myaungmya				0	B	w.	com.	com.						
Yodaya II	Myaungmya				0	D-E	w.	com.	com.						
Yodaya	Henzada				0	D	w.	com.	com.						
Yodayah	Kyaukpyu				0	F	w.	com.	l.b.						
Yodaya Kouk Nge	Bassein				0	D	w.	com.	com.						
Yodaya Ngasein	Pyapon				0	B	w.	com.	com.						
Yo-pyu	Myaungmya				0-10	A	w.	com.	w.	l.b.					
Ywet-taung	Myaungmya				0	B	w.	com.	com.						
Ywettaung	Bassein				0	A	w.	com.	com.						
Zale	Pegu				0	A-B	w.	com.	com.						
Zalun-pyu	Myaungmya				0	A-B	w.	com.	com.						
Zapawa-sangin	Maubin				0	B	w.	y.	y.						
Zaw-gyi-pyan	Myaungmya				0	A-B	w.	com.	l.b.						
Zaw-pyan	Pyapon				0	B	w.	com.	com.						
Zaw-tika	Pyapon				0	B	w.	com.	l.b.						

## Hulled grain

Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between		Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	Proportion between wts. of unhulled and hulled grain	Remarks	
				1	Thickness and Breadth									
6.52	2.41		2.71			a	com.	0						
6.43	2.47		2.60	d-f		com.	sl.							
6.20	3.05		2.03	a-b		com.	med.							
6.03	2.50		2.41	b		com.	sl.							
7.35	2.51		2.93	d		com.	sl.							
6.66	2.55		2.61	d		com.	cons.							
6.00	2.30		2.61	f		com.	sl.							Glumes oft. spotted with pur. b. color.
7.09	2.55		2.78	d		com.	sl.							
6.66	2.64		2.55	a		com.	sl.							
6.43	2.68		2.40	a		com.	med.							
6.58	2.70		2.44	b		com.	med.							
6.50	2.45		2.65	a		com.	sl.							
6.20	2.70		2.30	a-b		com.	sl.							
5.94	2.86		2.08	a-b		com.	cons.							
6.31	2.44		2.59	b		com.	sl.							
6.03	3.00		2.01	a-b		com.	med.							
6.00	2.70		2.22	b		com.	med.							
6.10	2.70		2.26	b		com.	med.							
														Abd. white Shiratama type.

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Weight of 100 grains	Specific gravity
							Empty glumes	Glumes	Awn		
Atin-Awoh	Kyaukpyu				0	C	w.	com.	com.		
Ayet-le-se	Akyab	8.20	2.94	2.10	0	B	w.	buff	l.buff	2.218	1.216
Bingala	Hemzada				0-15	B	b.	drab	b.	b. bl.	
Boobyank	Kyaukpyu				0	B	w.	com.+ pur.b.	l.b.		
Bu-thwin San Eyu	Sandoway				0	B	w.	com.	com.		
Da-Rai-Mee	Sandoway				0	E	w.	com.	com.		
E-Ma-Ata	Kyaukpyu				0	B	w.	com.	com.		
Goteto	Tharrawaddy				0	B	w.	com.	com.		
Htidawmo	Kyaukpyu				0	B	w.	com.	com.		
Hugat-pyawuway	N. Arakan				0	E	w.	com.	com.		
Kaubwe	N. Arakan	7.73	2.71	1.89	0	E	w.	com.	com.	1.992	
Kaukkyi-samaw	N. Arakan				0	E	w.	com.	com.		
Kaukkyi-Sanna	N. Arakan				0	E	w.	com.	com.		
Kaut-ya-gyi	Mergui				0	B	w.	com.	l.b.		
Kawdee	N. Arakan				0	B	w.	com.	com.		
Khunwa-gale	Maubin				0	B-D	w.	o.	o.		
Kun-ni	Myaungmya				0	B-D	w.	o.	o.		
Lakrun Sanpru	N. Arakan				0	F	w.	com.	l.b.		
Let-tow-ywe	Kyaukpyu				0	B	w.	l.y.g.	l.b.		
Lon-dat Kaukyin Myo	Bassein	8.11	2.74	2.12	0	D	w.	com.	com.	2.314	
Ma-lay-lok-lok	Pegu				0	B	w.	com.	com.		
Marabouk	Kyaukpyu				0	B	w.	com.	com.		
Mathalay	Pyapon				0	E	w.	com.	com.		
Mayin	Sandoway				0	B	w.	com.	com.		
Mayiwe-tot	Mergui				0	B	w.	com.	com.		
Namathalay Myathla-chaung	Maubin	8.8	2.38	1.83	0	E	w.	com.	com.		
Namathale	Pegu				0	E	w.	com.	com.	1.226	
Na-thalay	Tharrawaddy	6.15	2.27		0	E	w.	com.	com.	1.189	1.162

L.L.

N.-G., L., S.

Hulled grain											Remarks	
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between		Shape	Colour	Abdominal white	Volume of c.c.	Weight of g. 100 grains	Specific gravity	
6.25	2.25		2.78		c	com.	sl.	1.14	1.604	1.4070		
5.83	2.46	1.91	2.37	1.20	b	b.r.	med.	1.28	1.792	1.4000	808	
5.75	2.53		2.27		b	b.r.	cons.	1.37	1.912	1.3956	Glumes partly com. and partly pur. b. colored.	
6.00	2.46		2.44		b	com.	med.	1.35	1.896	1.4044		
5.83	2.57		2.27		b	com.	med.	1.37	1.896	1.3839		
6.00	2.46		2.44		e	com.	0	1.38	1.920	1.3913		
5.95	2.32		2.56		b	com.	0	1.23	1.708	1.3886		
4.90	2.40		2.04		b	com.	med.					
6.00	2.50		2.40		b	com.	med.	1.17	1.600	1.3675	Glumes spotted with pur. b. color.	
5.27	2.17		2.43		e	com.	0	0.93	1.356	1.4274		
5.93	2.37	1.64	2.50	1.45	e	com.	0	1.15	1.606	1.3965	806	
5.72	2.24		2.55		e	com.	0	0.94	1.316	1.4000		
4.91	2.04		2.41		e	b.r.	0	0.69	0.950	1.3768		
5.54	2.65		2.09		b	com.	med.					
5.10	2.12		2.41		b	com.	0	0.80	1.180	1.4750		
5.15	2.46		2.09		b-d	com.	0	1.14	1.594	1.3982		
6.28	2.35		2.67		b-d	com.	sl.					
5.47	2.24		2.44		f	com.	0	0.99	1.390	1.4040		
5.22	2.52		2.07		b	com.	sl.	1.23	1.692	1.3756	Glumes spotted with pur. b. color.	
6.16	2.34	1.76	2.63	1.33	d	com.	sl.		1.796		776	
6.05	2.47		2.45		b	com.	sl.					
5.65	2.57		2.20		b	com.	med.				Glumes spotted with b. color.	
4.80	1.90		2.53		e	com.	0					
5.80	2.50		2.32		b	com.	sl.					
5.76	2.55		2.26		b	com.	med.					
4.80	2.00		2.40		e	com.	0					
4.65	2.04	1.62	2.28	1.26	e	com.	0		0.954		778	
4.53	1.93	1.60	2.35	1.26	e	com.	0	0.66	0.952	1.4424	801	

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Awn	Tip of glumes
							Compl. glumes	Gleams	Awn		
Nga-sein-gyi	Tharrawaddy	8.21	2.96	1.95	0	B	w.	com.		l.b.	2.394
Nhit-la (2 months)	Akyab	8.22	2.90	2.05	0	B	w.	buff	l.buff	2.377	1.202
Nu-ma-thalay	Myaungmya	6.05	2.34	1.81	0	E	w.	com.	com.	1.234	1.162
Palaungbyu	Bassein				0	D	w.	com.	com.		
Palaungza	Kyaupkyu				0	E	w.	com.	com.		
Phwehlaung Mywe Saba	Tavoy				0	B	w.	com.	com.		
Phwehtaung-ni Saba	Tavoy				0	B	w.	l.b.	l.b.		
Pyn-thwe	Prome	8.70	2.96	1.98	0	B	w.	com.	com.	2.476	
Red paddy	N. Arakan	7.26	2.72	1.83	0-10	A-F	w.	buff	w.	b.	1.817
Sabar Ru Nge	Mergui				0	B	w.	com.	com.		
Sabani	Kyaupkyu				0	B	w.	l.o.	l.o.		
Sanni-gale	Sandoway				0	C	w.	com.	com.		
Sa-sa-Anet Kauk-yin-Myo	Bassein				0	E	b.	b.bl.	b.bl.		
Sa-sar	Pegu	6.29	2.34	1.81	0	E	b.	dar.b.	dar.b.	1.286	1.155
Satahaung	N. Arakan				0	E	w.	com.	com.		
Sa-tha-Saba	Myaungmya				0	E	b.	b.bl.	b.bl.		
Sat-sa-ba	Myaungmya	6.33	2.28	1.77	0	E	w.	com.	com.	1.249	1.153
Saza	Toungoo	8.17	3.24	2.08	0-13	B	w.	buff	w.	com.	2.546
Sit-sa-gaung	Tharrawaddy	8.43	2.76	2.09	0	B	w.	l.o.	l.o.	2.497	
Small paddy	N. Arakan	7.21	2.57	1.72	0	E	w.	com.	com.	1.540	
Sunlet-the Kauk-let	Sandoway	7.98	3.10	1.93	0	C	w.	com.	com.	2.109	1.154
Taungsaint	N. Arakan				0	E	w.	o.	com.		
Uundun	N. Arakan	6.78	2.38	1.66	0	E	w.	com.	ccm.	1.290	1.216
Ya-tha-le Kauk-yin	Bassein	6.23	2.37	1.85	0	E	w.	com.	com.	1.235	
Ya-tha-le Sapa	Tharrawaddy	6.08	2.39	1.82	0	E	w.	com.	com.	1.304	1.176
Ye-gyoon Saba	Kyaupkyu				0	B	w.	com.	com.		
Ywatshok	Sandoway				0	B	w.	o.	o.		

Hulled grain												Remarks	
Length m.m.	Breadth m.m.	Thickness m.m.	Proportion between Breadth and Length Thickness and Breadth		Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	Proportion between wts. of unhulled and hulled grain		
			Breadth	Length									
5.96	2.43	1.71	2.45	1.42	b	com.	sl.		1.877		784	Glumes lighter colored toward base & on veins.	
5.77	2.47	1.86	2.34	1.33	b	l.b.r.	med.	1.36	1.910	1.4059	804		
4.62	1.99	1.57	2.32	1.27	e	com.	0	0.70	0.983	1.4043	797		
6.29	2.29		2.75		d	com.	sl.						
4.90	2.20		2.23			com.	0						
6.21	2.40		2.59		b	com.	med.						
5.50	2.33		2.36		b	com.	sl.						
6.38	2.49	1.73	2.56	1.44	b	com.	sl.		1.916		774		
5.32	2.38	1.70	2.24	1.40	a-f	l.b.r.	0	1.03	1.445	1.4029	795		
6.05	2.33		2.59		b	com.	sl.						
6.10	2.43		2.51		b	com.	sl.						
5.96	2.35		2.54		c	b.r.	sl.						
5.88	2.10		2.80		e	com.	0						
4.67	2.02	1.57	2.31	1.29	e	com.	0	0.70	0.993	1.4185	772		
5.94	2.42		2.45		e	com.	0						
4.90	2.10		2.33		e	com.	0						
4.71	1.98	1.56	2.38	1.27	e	com.	0	0.70	0.984	1.4057	788	Glumes lighter colored on veins	
5.85	2.56	2.00	2.28	1.28	b	b.r.	sl.	1.42	2.005	1.4120	788		
6.37	2.36	1.83	2.70	1.28	b	com.	sl.		1.939		776		
5.37	2.23	1.55	2.41	1.44	e	b.r.	0		1.226		796		
5.94	2.48	1.67	2.39	1.49	c	com.	sl.	1.23	1.676	1.3626	794		
6.14	2.32		2.65		c	com.	sl.					Glumes oft. spotted with pur. b. color.	
5.12	2.00	1.50	2.45	1.39	e	b.r.	0	0.74	1.034	1.3973	802		
4.64	2.05	1.59	2.26	1.29	e	com.	0	0.69	0.986	1.4289	800		
4.66	2.04	1.61	2.28	1.27	e	com.	0	0.73	1.041	1.4260	798		
6.14	2.42		2.54		b	com.	med.						
6.40	2.30		2.81		b	com.	sl.						

SHORT-G

LAR

## RAINED.

G E .

N.-G., S., L.

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length m.m.	Proportion between Thickness and Breadth	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	
5.92	3.08		1.92		b	com.	cons.	1.82	2.456	1.3495	Glumes streaked with l.b. color. Abd. w. Shiratama type.
5.85	3.10	2.20	1.89	1.41	a	com.	cnos.	1.94	2.618	1.3495	
6.16	3.15	2.08	1.96	1.51	a-b	com.	cons.	1.83	2.534	1.3847	792
5.90	3.25	2.08	1.82	1.56	a-b	com.	med.	1.81	2.460	1.3591	807
5.75	3.14		1.83		b	com.		1.84	2.532	1.3761	
5.70	3.25	2.10	1.75	1.55	a-b	com.	cons.	1.70	2.372	1.3953	793
5.77	3.15		1.64		a-b	com.	cons.				
6.00	3.12		1.92		a-b	com.	cons.	1.87	2.592	1.3861	
5.92	3.42		1.73		a-b	com.	cons.				
5.65	3.35		1.69		f	com.	med.				Glumes oft. Streaked with b. color.
5.67	3.18	2.23	1.78	1.43	b	com.	med.		2.561		801
6.00	3.00		2.00		a-b	com.	cons.				
5.90	3.10		1.90		a-b	com.	med.	1.76	2.446	1.3899	Abd. white Shiratama type.
5.80	3.20		1.81		b	com.	med.				
5.85	3.10		1.89		b	com.	med.	1.87	2.588	1.3840	
6.00	3.10		1.94		a-b	com.	med.				
5.95	3.10		1.92		a	com.	med.	1.89	2.644	1.3989	
6.00	3.10		1.94		a	com.	med.	1.84	2.564	1.3935	
6.00	3.10		1.94		b	com.	med.	1.75	2.474	1.4137	
6.00	3.10		1.94		b	com.	med.	1.80	2.536	1.4089	
5.64	2.99		1.89		a-b	com.	med.				Glumes streaked with b. color.
5.90	3.20		1.84		b	com.	cons.	2.06	2.836	1.3767	Glumes oft. Streaked with o. color.
6.13	3.19	2.13	1.92	1.49	b	com.	cons.	1.97	2.720	1.3807	795
5.80	3.15		1.84		a-b	com.	med.	1.94	2.701	1.3923	Glumes oft. Streaked with o. color.
5.95	3.03		1.96		a-b	com.	med.	1.78	2.466	1.3854	
6.10	3.29	2.17	1.86	1.52	b	com.	med.	2.04	2.863	1.4034	800
											Abd. white Shiratama type.

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of				Specific gravity
							Empty glumes	Glumes	Awn	Tip of glumes	
Midon-Kaukyi	Hemzada				0	B	l.b.	com.		l.b.	
Minthagale	Maubin				0	A	w.	com.		com.	
Myan-Aung Gopa	Pyapon				0-18	B	l.b.	com.	l.b.	l.b.	
Nga-Kywe Mye-she	Thaton				5-50	B	l.b.	l.b.	l.b.	b.	
Ngakywe Saba	Myaungmya				0-15	B	w.	drab	l.b.	drab	
Paw Bin chaung	Pyapon				0-5	B	w.	com.	l.b.	com.	
Pawa	Sandoway				0	B	w.	com.		com.	
Sa Ba Myishe	Thaton				0-30	A-B	b.	0	b.	b.	
Sadokepyat	Myaungmya				0	A	w.	com.		l.b.	
Sapanet Mi So	Pyapon				0-25	B	w.	drab	b.	b.	
Sapa Net	Myauugmya	7.97	3.67	2.47	0-20	B	w.	drab	b.	b.	3.274 1.203
Sapawa	Pegu				0	B	b.	l.o.		b.	
Sapawa-mi	Pyapon	8.22	3.61	2.31	0-15	B	w.	com.	b.	b.	3.336
Sathawa Meso	Pyapon				0-35	B	w.	com.	l.b.	b.	
Shwe-deik Soe	Myaungmya				0-12	B	l.b.	com.	l.b.	b.	
Shwelaung-gyi	Myaungmya				0	B	w.	com.		l.b.	
Shwe-wa	Thaton				10-45	B	w.	com.	w.	com.	
Thee-dat	Salween				0	F	w.	com.		b.	

## M E D

Ah-nee	Myaungmya	7.32	3.55	2.46	0-20	B	l.b.	com.	l.b.	l.b.	
Baw youk-ke	Kyaukpyu	7.57	3.38	2.39	0-20	B	l.b.	com.	l.b.	l.b.	2.580 1.211
Bawyut	Myaungmya	7.40	3.42	2.27	0-15	B	w.	com.	l.b.	com.	3.050
Bawyut	Sandoway				0-5	B	w.	com.	b.	b.	
Bawyut gwado	Pyapon				0	B	w.	com.		l.b.	
Bawyut-meshe	Pyapon				0-15	B	l.b.	com.	l.b.	l.b.	
Bawyut-miso	Pyapon				0-15	B	w.	com.	l.b.	l.b.	
Bein Datrek	Kyaukpyu				0-15	B	w.	com.	b.	b.	

## Hulled grain

Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of c.c.	Weight of 100 grains g.	Specific gravity	Proportion between wts. of unhulled and hulled grain	Remarks
5.90	3.20			1.84	b	com.	cons.	1.90	2,618	1.3779		
5.90	3.24			1.82	a	com.	cons.					
5.93	3.05			1.94	b	com.	med.	1.77	2,470	1.3955		
5.97	3.05			1.96	b	com.	med.					
6.06	3.07			1.97	b	com.	med.	1.76	2,254	1.2807		
6.10	3.13			1.95	b	com.	med.	1.75	2,450	1.4000		
5.92	3.05			1.94	b	com.	cons.					
6.05	3.13			1.93	a-b	com.	enos.					
5.89	3.35			1.76	a	com.	med.					
5.80	3.19			1.82	b	com.	med.					
5.95	3.20	2.16	1.86	1.48	b	com.	med.	1.86	2,604	1.4000	795	
6.05	3.05			1.98	b	com.	med.					
5.97	3.10	2.02	1.93	1.53	b	com.	med.					
6.00	3.10			1.94	b	com.	med.					
6.00	3.10			1.94	b	com.	med.					
6.02	3.02			1.99	b	com.	med.					
5.95	3.10			1.92	b	com.	med.					
5.73	3.25			1.76	f	com.	med.					

## I U M.

5.60	3.00	2.16	1.87	1.39	b	com.	sl.	1.74	2,428	1.3954		Abd. white Shiratama type.
5.69	3.01	2.10	1.89	1.43	b	com.	cons.	1.53	2,142	1.4000	830	
5.62	3.03	2.02	1.85	1.50	b	com.	cons.	1.78	2,452	1.3775	804	
5.70	3.10		1.84		b	com.	med.					
5.75	2.98		1.93		b	com.	med.	1.77	2,474	1.3977		
5.82	3.00		1.94		b	com.	med.	1.81	2,536	1.4011		Abd. white Shiratama type.
5.64	2.96		1.91		b	com.	med.	1.69	2,348	1.3893		Abd. white Shiratama type.
5.70	3.00		1.90		b	com.	med.	1.74	2,396	1.3770		

Variety	District	Unhulled grain										Specific gravity	
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Awn	Tip of glumes		
							Empty glumes	Glaunes	Awn				
Bootkwa Saugui	Maubin				0	B	w.	com.		l.b.			
Bu-Byauk Kaukkyi	Sandoway				0	B	l.b.	l.o.		b.			
Byat	Myaungmya				0	B	w.	com.		com.			
Byat Kaukkyi	Hemzada				0	B	w.	com.		l.b.			
Byat Pu	Pegu				0-10	B	w.	com.	l.b.	l.b.			
Byat-Saba	Myaungmya				0	B	l.b.	com.		b.			
Byatwa	Sandoway				0	B	w.	com.		l.b.			
Da-Le-San	Pegu				0	B	l.b.	com.		l.b.			
Dalisan	Toungoo	7.27	3.49	2.34	0-10	B	w.	com.	l.b.	l.b.		1.219	
Dalisan	Kyaukpyu				0	B	w.	com.		b.			
Dalisangne	Toungoo	7.14	3.52	2.47	0-10	B	w.	com.	w.	com.		2.646	
Eik-Bal Longtal-Saba	Tavoy				0-15	B	w.	com.	w.	l.b.			
Kaub-byi Nga-bywe	Tharrawaddy				0-10	B	w.	drab	l.b.	b.			
Kamakyi	Myaungmya				0-18	A	b.	com.	b.	b.			
Kaub-byi-Ni	Tharrawaddy				0-15	B	b.	com.	b.	b.			
Kaukaw	N. Arakan	7.47	3.46	2.38	0-10	B	w.	com.	w.	com.			
Kauk-kyi	Toungoo				0	B	b.	y		b.			
Kauk-kyi Ahbaungne	Myaungmya				0-20	B	l.b.	l.y.	l.b.	b.			
Kauk-kyi Byu	Sandoway	7.68	3.69	2.40	0-12	B	w.	com.	l.b.	com.		1.170	
Kauk-kyi Meshe	Pyapon				0-15	B	w.	com.	l.b.	l.b.			
Kauk-kyi Sabase	Sadoway	7.38	3.49	2.25	0	B	l.b.	com.		l.b.	2.685	1.209	
Kauk-kyi Thee-dat	Myaungmya				0	A-B	w.	com.		com.			
Kauk-san	Myaungmya	7.47	3.52	3.36	0	B	l.b.	com.		l.b.	2.936		
Kauk-ya	Myaungmya	7.16	3.62	2.43	0	A-B	w.	com.		com.	2.868		
Kaukgyi	Myaungmya	7.55	3.78	2.34	0	B	w.	com.		com.	3.025	1.195	
Khun Ni Sapa	Pyapon				0-10	B	l.b.	o.	l.b.	o.			
Koe-mai-Sin	Myaungmya				0	B	w.	com.		com.			
Komai Sapa	Pyapon				0	B	w.	com.		com.			
Kyauk Sangyi	Hemzada				0	B	w.	com.		com.			
Kyaukgyi	Myaungmya				0	B	w.	com.		l.b.			

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of 100 grains c. c.	Weight of 100 grains g.	Specific gravity	
5.80	3.10		1.87		b	com.	med.				
5.70	2.90		1.97		b	com.	med.				
5.70	3.00		1.90		b	com.	med.	1.66	2.338	1.4084	
5.58	3.10		1.80		b	com.	cons.	1.77	2.428	1.3718	
5.80	3.00		1.93		b	com.	cons.	1.64	2.290	1.3963	
5.80	3.10		1.87		b	com.	med.	1.82	2.530	1.3901	
5.50	3.05		1.80		b	com.	med.	1.64	2.240	1.3659	
5.60	3.10		1.81		b	com.	cons.	1.77	2.418	1.3661	
5.75	3.00	2.04	1.92	1.47	b	com.	med.	1.63	2.284	1.4012	
5.70	3.00		1.90		b	com.	med.				
5.63	3.06	2.13	1.84	1.44	b	com.	med.	1.58	2.170	1.3734	802
5.50	3.05		1.80		b	com.	med.	1.76	2.454	1.3943	
5.90	3.00		1.97		b	com.	cons.	1.71	2.370	1.3860	
5.72	3.05		1.88		a	com.	med.	1.69	2.346	1.3882	
5.90	3.00		1.97		b	com.	cons.	1.79	2.450	1.3687	
5.74	3.01	2.12	1.91	1.42	a	com.	med.	1.69	2.390	1.4142	
5.80	3.10		1.87		b	com.	med.	1.66	2.318	1.3964	
5.80	3.00		1.93		b	com.	med.	1.73	2.400	1.3873	Glumes streaked with r. b. color.
5.50	3.00	2.15	1.83	1.40	b	com.	med.	1.63	2.222	1.3632	
5.90	3.00		1.97		b	com.	med.	1.77	2.472	1.3966	
5.50	3.00	2.00	1.86	1.50	b	com.	sl.	1.52	2.124	1.4000	791
5.40	3.15		1.71		a	com.	med.	1.67	2.332	1.3964	Abd. white Shiratama type.
5.76	3.03	2.08	1.90	1.46	b	com.	med.	1.68	2.334	1.3893	795
5.58	3.09	2.08	1.81	1.49	a	com.	med.	1.62	2.286	1.4112	797
5.80	3.28	2.08	1.77	1.58	b	com.	med.	1.74	2.440	1.4023	807
5.80	3.00		1.93		b	com.	med.	1.72	2.412	1.4023	Glumes lighter color- ed on veins.
5.90	3.00		1.97		b	com.	sl.	1.65	2.282	1.3830	
5.90	3.00		1.97		b	com.	med.	1.68	2.358	1.4036	Glumes lighter color- ed on veins.
5.70	3.00		1.90		b	com.	cons.	1.65	2.284	1.3842	
5.70	2.90		1.97		b	com.	med.	1.46	2.044	1.4000	Abd. white Shiratama type.

Variety	District	Unhulled grain										Specific gravity	
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Tip of glumes	Weight of 100 grains		
							Empty glumes	Glumes	Awn				
Kyien-thee	Sandoway				0	B	b.	com.		b.			
Kyein-thee gangyaung }	Maubin				0	A	w.	com.		com.			
Kye-ne young	Myaungmya				0-6	A	l.b.	com.	l.b.	b.			
Kyi-ni	Myaungmya				0	B	l.b.	com.		l.b.			
Kyi-ni-young	Tharrawaddy				0-5	A	l.b.	com.	b.	b.			
Kyoukproukunantha	N. Arakan	6.89	3.47	2.49	0-4	B	l.b.	com.	b.	b.	2.576		
Lon-Bu-Myi-she	Thaton				20-40	B	w.	com.	w.	com.			
Lonbu Saba	Tavoy				0-25	B	w.	com.	l.b.	l.b.			
Loongyi	Kyaukpyu				0	B	l.b.	o.		b.			
Loonphyu	Akyab				0	A-B	w.	com.		com.			
Me Nyet	Pyapon				0-20	B	w.	drab		drab			
Mi-don Kaukyit	Pyapon				0	B	w.	com.		l.b.			
Myatpongyi	Hemzada				0	B	w.	com.		l.b.			
Nat-kun-sin	Myaungmya				0	B	w.	com.		com.			
Nga Kyinthe Byu	Sandoway				0	B	w.	l,drab		b.			
Nga Kyini	Myaungmya				0	B	l.b.	o.		b.			
Nga-kywe	Hemzada				0-20	B	w.	drab	l.b.	b.			
Nga-kywe I	Myaungmya				0	B	w.	drab		b.			
Nga-kywe II	Myaungmya				0-10	B	w.	drab	b.	b.			
Nga-kywe	Sandoway				0-20	B	w.	drab	b.	b.			
Nga-kywe III	Myaungmya				0-15	B	w.	drab	b.	b.			
Nga-kywe IV	Myaungmya				0-20	B	w.	drab	b.	b.			
Nga-kywe	Pyapon				0	B	w.	drab		b.			
Nga-kywe-gale I	Myaungmya				0-10	B	w.	drab	b.	b.			
Nga-kywe-gale II	Myaungmya				0-20	B	w.	drab	b.	b.			
Nga-kywe-kale	Myaungmya				0-15	B	w.	drab	b.	b.			
Nga-kywe-midon	Pegu				0-10	B	w.	drab	l.b.	b.			
Nga-kywe Mwe	Myaungmya				0-30	B	w.	drab	l.b.	b.			
Nga-kywe Pyu	Myaungmya				0	B	l.b.	com.		l.b.			
Nga-kywe Pyu II	Myaungmya				0	B	w.	com.		com.			

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of c. c. 100 grains	Weight of g. 100 grains	Specific gravity	
5.90	3.05		1.93		b	com.	cons.	1.80	2.498	1.3878	
5.80	3.10		1.87		a	com.	med.	1.84	2.576	1.4000	
5.79	3.09		1.87		a	com.	med.	1.74	2.404	1.3816	
5.90	3.00		1.97		b	com.	sl.	1.66	2.322	1.3988	
5.85	3.04		1.92		a	com.	med.				
5.52	3.23	2.19	1.71	1.48	b	com.	med.				Glumes streaked with l.b.r. color.
5.80	3.10		1.87		b	com.	med.				
5.82	3.05		1.91		b	com.	med.				
5.80	3.00		1.93		b	com.	med.				Glumes partly lighter colored.
5.60	3.00		1.87		a	com.	med.				
5.85	3.05		1.92		b	com.	cons.				
5.70	3.10		1.84		b	com.	sl.				
5.80	3.07		1.89		a	com.	cons.				
5.76	2.90		1.99		b	com.	cons.	1.61	2.214	1.3752	
5.80	3.10		1.87		b	com.	cons.				
5.84	3.00		1.95		b	com.	cons.				
5.80	3.00		1.93		b	com.	cons.				
5.80	3.10		1.87		b	com.	cons.				
5.75	2.97		1.94		b	com.	med.				
5.85	3.00		1.95		b	com.	cons.				
5.60	3.10		1.81		b	com.	cons.				
5.75	3.03		1.90		b	com.	cons.				
5.77	3.05		1.89		b	com.	med.				
5.80	3.02		1.92		b	com.	cons.				
5.70	3.00		1.90		b	com.	cons.				
5.88	3.05		1.93		b	com.	med.				
5.86	3.02		1.97		b	com.	med.				
5.70	3.10		1.84		b	com.	med.				
5.70	2.95		1.93		b	com.	cons.				
5.70	2.98		1.91		b	com.	cons.				

Proportion between  
wts. of unhusked  
and hulled  
grain

Variety	District	Unhulled grain										Specific gravity	
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Tip of glumes	Weight of 100 grains		
							Empty glumes	Glumes	Awn				
Nga-Kywo Dawebyu	Maubin				0-12	B	w.	drab	l.b.	b.			
Ngalu	Toungoo				0	B	w.	com.		com.			
Ngasein-gyi	Pegu				0	B	l.b.	com.		com.			
Nga-wa-gyi	Pegu				0	B	w.	com.		b.			
Ngayabo	Toungoo				0	B	w.	com.		com.			
Fa-go-Ngasein	Pyapon				0	B	w.	com.		com.			
Pan-pin-chaung	Myaungmya				0-20	B	w.	com.	l.b.	b.			
Pan-pin-chaung	Pegu				0	B	w.	com.		l.b.			
Pok-gyi	Myaungmya	7.45	3.61	2.43	0	A	w.	com.		com.	3.081		
Pu-nyo	Myaungmya				0-20	B	w.	com.	w.	l.b.			
Saba Ma	Pyapon				0-20	B	w.	com.	w.	com.			
Saba-Net-Myishe	Thaton				0-45	B	w.	drab	b.	b.			
Saba-Net Nagywe	Pegu	7.67	3.58	2.32	0-20	B	w.	drab	l.b.	b.	3.060	1.201	
Sabanet Saba	Pyapon				0-10	B	w.	drab	l.b.	b.			
Sa-pa-net Midon	Pegu				0-5	B	w.	drab	l.b.	b.	3.169	1.207	
Sapa Net Midon	Pyapon				0-30	B	w.	drab	l.b.	b.			
Sein-ta Waut	Myaungmya				0	B	l.b.	com.		b.			
Shwe-deik Soe	Myaungmya				0-30	B	w.	com.	l.b.	b.			
Shwe-wa-gyi	Myaungmya				0-30	B	w.	com.	l.b.	l.b.			
Tanlwe	Kyaukpyu				0-10	B	w.	com.	w.	l.b.			
Thee-dat	Myaungmya				0	B	w.	com.		b.			
Thon-byando Ngakywe }	Myaungmya				0-10	B	w.	drab	b.	b.			
Tobwagyi	Pyapon				0	B	w.	l.y.		l.b.			
Zaw-pyan-gyi	Myaungmya				0	B	w.	com.		l.b.			
Zebaw-we	Henzada				0	B	w.	com.		l.b.			

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Proportion between Breadth and Length Thickness and Breadth		Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	
											Proportion between wts. of unhulled and hulled grain
5.55	2.98		1.86		b	com.	med.	1.71	2.370	1.3860	
5.70	3.10		1.84		b	com.	med.				
5.75	3.10		1.85		b	com.	cons.				
5.77	3.00		1.92		a	com.	med.				
5.92	3.00		1.97		b	com.	med.				
5.75	3.04		1.89		b	com.	med.				
5.77	3.00		1.92		b	com.	cons.				
5.80	3.10		1.87		b	com.	med.				
5.54	3.19	2.19	1.74	1.46	a	com.	med.		2.447		794
5.78	3.02		1.91		b	com.	med.				
5.70	3.00		1.90		b	com.	med.				
5.70	3.10		1.84		b	com.	med.				
5.73	3.06	2.09	1.87	1.46	b	com.	med.	1.72	2.410	1.4012	788
5.90	3.00		1.97		b	com.	med.				
5.70	3.05		1.87		b	com.	sl.	1.76	2.485	1.4119	784
5.66	3.09		1.83		b	com.	cons.				
5.58	3.05		1.83		b	com.	cons.				
5.55	3.10		1.79		b	com.	cons.				Abd. white Shiratama type.
5.55	3.16		1.76		b	com.	sl.				Abd. white Shiratama type.
5.85	3.00		1.95		b	com.	cons.				
5.75	3.00		1.92		b	com.	sl.				Abd. white Shiratama type.
5.65	2.97		1.90		b	com.	med.				
5.90	3.05		1.93		b	com.	med.				
5.95	3.00		1.98		b	com.	cons.				
5.70	3.11		1.83		a-b	com.	cons.				

## SMA

Variety	District	Unhulled grain										Specific gravity	
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Awn	Tip of glumes		
							Empty glumes	Glumes	Awn				
Baw-yut Medo	Myaungmya				0-10	B	w.	com.	w.	1.b.			
Gauk-gyi-kale	Myaungmya				0	B	1.b.	com.		b.			
Hadawa	Myaungmya	7.30	3.35	2.30	0-4	B	1.b.	com.	1.b.	b.	2.582	1.184	
Kye Byu	Sandoway				0-20	B	1.b.	com.	1.b.	1.b.			
Mi-don-laung	Pegu				0	B	w.	drab		b.			
Nga-sain	Myaungmya				0	B	1.b.	com.		b.			
Pale-pyan	Myaungmya				0-20	B	1.b.	com.	b.	b.			
Phalaung Nga-kwe	Sandoway	5.78	2.77	1.92	0	B	b.	b.bl.		b.bl.		1.154	
Pindo	Henzada				0	B	w.	com.		com.			
Pusochon	Henzada				0	B	w.	com.		1.b.		1.146	
Sabaze	Kyaukpyu	7.00	3.27	2.30	0	B	1.b.	com.		b.	2.425	1.183	
Satha Saba	Kyaukpyu				0	B	b.	b.bl.		b.bl.			
Shwedeik-pwa	Myaungmya				0-20	B	w.	com.	1.b.	1.b.			
Shweda Di	Kyaukpyu	6.12	3.10	2.24	0-15	A	1.b.	com.	1.b.	b.		1.211	
Tawng-gyi	Toungoo				0	A-B	1.b.	com.		b.			
Yema-naing	Myaungmya	7.26	3.44	2.45	0-10	B	w.	com.	1.b.	b.			

L L.

N.-G., S., s.

Hulled grain												Remarks	
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length	Proportion between Thickness and Breadth	Shape	Colour	Abdominal white	Volume of c.c. 100 grains	Weight of 100 grains	Specific gravity	Proportion between wts. of unhulled and hulled grain		
5.20	3.08		1.69		b	com.	med.	1.66	2.316	1.3952		Abd. white Shiratama type.	
5.45	2.95		1.85		b	com.	med.	1.60	2.242	1.4013		Abd. white Shiratama type.	
5.60	2.89	2.00	1.94	1.45	b	com.	sl.	1.49	2.070	1.3892	802		
5.52	2.94		1.88		b	com.	sl.	1.69	2.358	1.3953		Long glumed.	
5.36	2.85		1.88		b	com.	cons.						
5.60	2.90		1.93		b	com.	cons.	1.58	2.202	1.3937			
5.50	2.80		1.96		b	com.	med.	1.54	2.174	1.4117			
4.24	2.38	1.70	1.78	1.40	b	l.gr.	sl.	0.68	0.962	1.4147		Abd. white Shiratama type.	
5.45	2.80		1.95		b	com.	med.	1.50	2.088	1.3920			
5.60	2.90	2.02	1.93	1.44	b	com.	med.	1.40	1.930	1.3786			
5.27	2.80	2.06	1.88	1.36	b	com.	med.	1.40	1.926	1.3757	794		
4.20	2.45		1.71		b	com.	sl.	0.78	1.082	1.3872			
5.20	3.00		1.73		b	com.	med.	1.60	2.230	1.3938			
4.45	2.74	2.00	1.62	1.37	a	com.	sl.	1. 0	1.546	1.4055			
5.50	2.90		1.90		a-b	com.	cons.	1.57	2.172	1.3834			
5.48	2.94	2.15	1.86	1.57	b	com.	med.	1.64	2.292	1.3976			

GLUT  
SLENDER  
LAR

Variety	District	Unhulled grain										Specific gravity
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Empty glumes	Glumes	Awn	Tip of glumes	Weight of 100 grains	
Ka-Baing	Sandoway				0	C	w.	b.		b.		
Kaung-nyin Sin-do	Myaungmya				0-15	C-D	w.	b.	l.b.	b.		
Kyaw-ne	Myaungmya	10.04	3.06	2.13	0-16	C-D	w.	o.	l.b.	b.		1.147
Kyit-phi Me	Mergui				0-15	D	w.	com.	w.	com.		
Mingyilatyon	Bassein	10.77	3.06	2.27	0	D	w.	com.		com.	3.583	1.155
Nga Yantha-baub	Pyapon				0	C	w.	b.		l.b.		
Nga-pyaw-Nyun	Myaungmya	10.91	3.08	2.19	0	C-D	w.	com.		com.	3.364	1.147
Nga-pyaw Nyun	Pegu	10.76	3.09	2.17	0	C	w.	com.		com.	3.132	1.150
Nyaing-gaing	Sandoway	10.91	2.86	2.24	0	D	l.b.	b.		b.	3.073	1.139
Paukwagyi	Hemzada				0	C	w.	com.		com.		
Sein Byu Kauk Hiyin	Thaton	10.58	3.43	2.30	0	D	w.	com.		com.	3.418	1.161
Sepaung	Pegu	10.91	3.03	2.21	0	D	w.	com.		com.	3.443	1.153
Shwekyi-dauk	Hemzada				0	C	w.	com.		com.		
Shwe-Kya Dauk	Sandoway	10.65	3.07	2.22	0	C	w.	com.		com.	3.557	1.156
Shwe Kyi Dauk	Pegu	10.87	3.13	2.13	0	C	w.	com.		com.	3.469	1.143
Shwelando	Myaungmya				0-20	D	l.b.	b.	l.b.	b.		
Shwethwe	Hemzada				0	C-F	w.	com.		pur.b.		
Wetto-plan	Myaungmya				0-20	F	l.b.	l.b.	l.b.	b.		
Winkaung	Toungoo				0	F	w.	com.		com.		
Zaw-Nyun	Bassein	10.93	3.14	2.19	0	D	w.	com.		com.	3.557	1.157

## INOUS RICE.

## GRAINED.

GE.

G., S., I.

Hulled grain										Remarks	
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length	Proportion between Thickness and Breadth	Shape	Colour	Abdominal white	Volume of 100 grains c. c.	Weight of 100 grains g.	Specific gravity	
										Proportion between wts. of unhulled and hulled grain	
8.14	2.46		3.31		c	t.c.					
7.74	2.57		3.01		c-d	w.		1.68	2.356	1.4024	
7.65	2.54	1.90	3.01	1.34	c-d	w.		1.64	2.278	1.3890	
7.64	2.48		3.08		d	w.		1.73	2.426	1.4023	
8.03	2.46	1.98	3.26	1.24	c	w.		1.98	2.788	1.4081	778
7.50	2.50		3.00		c	w.					
8.05	2.61	1.92	3.08	1.36	c-d	w.		1.92	2.690	1.4010	800
7.84	2.52	1.97	3.11	1.28		w.		1.72	2.380	1.3837	760
7.88	2.43	1.95	3.24	1.25	d	w.		1.71	2.412	1.4105	785
8.00	2.66		3.01		c	w.		1.90	2.704	1.4232	
7.95	2.56	2.05	3.11	1.25	c	w.		1.93	2.700	1.3990	790
8.07	2.36	1.92	3.42	1.23	c	w.		1.86	2.597	1.3962	754
7.85	2.61		3.01		c	w.		1.83	2.544	1.3902	
8.02	2.46	1.92	3.26	1.28	c	w.		1.93	2.753	1.4264	774
8.13	2.36	1.88	3.44	1.26	c	w.		1.89	2.662	1.4085	767
7.74	2.55		3.04		d	w.		1.73	2.426	1.4023	
7.76	2.44		3.18		c-f	w.					Glumes partly streaked with pur. b. color.
7.60	2.50		3.04		f	w.		1.67	2.326	1.3928	
7.91	2.52		3.14		c-f	w.		1.68	2.354	1.4012	
8.22	2.50	1.83	3.29	1.37	d	w.		1.97	2.789	1.4157	784

## MED

Variety	District	Unhulled grain										
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Empty glumes	Glumes	Awn	Tip of glumes	Weight of 100 grains g.	Specific gravity
Ahgat	Sandoway	10.52	2.81	2.19	0	D	w.	com.		b.	3.274	1.157
Ait-tharu	Pegu				0-10	D	w.	com.	w.	com.		
Eik-thun Byu	Pyapon				0	D	w.	com.		com.		
Eik-thun-ni	Pyapon	9.94	2.84	2.05	0	D	w.	l.o.		b.	2.709	1.146
Hlay-w-swe	Myaungmya				0	D	w.	com.		com.		
Kaukyin Weh Thasi	Pyapon				0-10	C-D	w.	com.	w.	com.		
Kabaing Kaung Hyin	Sandoway				0	D	w.	l.b.		l.b.		
Kaukyin Shwe Hlau Do	Pyapon				0-10	C-D	w.	b.	l.b.	b.		
Kaukyin Taung- gyaw	Myaungmya	10.13	2.59	2.09	0	C-D	w.	com.		com.		1.147
Kawng-nyin Kyu-ban	Myaungmya				0	D	w.	com.		l.b.		
Kaung-Nyin	Myaungmya				0-9	D	w.	l.o.	w.	l.o.		
Kaung-nyin Kywe- thwa	Myaungmya				0	C	l.b.	b.y.		dar.b.		
Kyaw-ha	Myaungmya	10.31	2.65	2.09	0	D	w.	o.		l.o.		1.143
Kyaw-hoe	Myaungmya	10.39	2.76	2.11	0	D	w.	o.		l.o.		1.145
Kyaung Hnyin	Pyapon				0	D	w.	b.		b.		
Kyawho	Bassein	10.09	2.72	2.11	0	D	w.	o.		l.o.	2.746	1.144
Kyaw singale	Hemzada				0	D	w.	l.o.		b.		
Kyaw-Ho	Sandoway				0	C	w.	b.		l.b.		
Kyaw-Zin	Sandoway				0	D	w.	com.		l.b.		
Kyetpami	Toungoo	10.22	2.98	2.19	0	F	w.	com.		com.		1.145
Kyuban	Myaungmya	9.84	2.93	2.15	0	D	w.	com.		com.		1.131
Kyuban	Sandoway	10.31	2.84	2.07	0	D	w.	com.		com.	2.925	1.170
Kyupan	Tharrawaddy				0-3	C	w.	l.o.	w.	b.		
Kywet-thwa	Myaungmya	10.17	2.90	2.13	0	D	w.	com.		com.		1.147
Lin-ma-ne	Myaungmya				0	D-F	w.	com.		com.		
Lin-mane Kauk Hnyin	Pyapon				0-5	D	w.	b.	l.b.	b.		
Ma-IImin-Nywe	Pavoy	10.24	2.58	2.09	0	D	w.	y.		b.	2.770	1.155
Mein-mahla Kauk Hnyin	Pyapon				0	D	w.	l.o.		b.		

I U M .

G., S., M.

Hulled grain											Remarks	
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between Thickness and Breadth	Shape	Colour	Abdominal white	Volume of 100 grains e.e.	Weight of 100 grains g.	Specific gravity		
7.80	2.35	1.95	3.32	1.21	d	w.		1.82	2.573	1.4137	786	
7.08	2.34		3.03		d	w.		1.54	2.154	1.3987		
7.17	2.32		3.09		c-d	w.		1.59	2.238	1.4075		
7.49	2.21	1.82	3.30	1.21	d	w.		1.54	2.168	1.4078	800	
7.14	2.38		3.00		d	w.		1.50	2.104	1.4027		
7.50	2.40		3.13		c-d	w.		1.60	2.258	1.4113		
7.21	2.34		3.08		d	t.c.		1.33	1.870	1.4060		
7.28	2.42		3.01		c-d	w.		1.60	2.240	1.4000		
7.45	2.26	1.82	3.30	1.24	c-d	w.		1.53	2.144	1.4013		
7.30	2.43		3.00		d	w.		1.51	2.130	1.4106		
7.31	2.39		3.06		d	w.		1.52	2.134	1.4039		
7.21	2.36		3.06		c	w.		1.47	2.070	1.4082		
7.43	2.27	1.82	3.27	1.25	d	w.		1.46	2.026	1.3877		
7.32	2.33	1.89	3.14	1.23	d	w.		1.43	2.016	1.4098		
7.09	2.36		3.00		d	w.		1.57	2.174	1.3847		
7.58	2.16	1.87	3.51	1.16	d	w.		1.52	2.131	1.4020	776	
7.48	2.45		3.05		d	w.		1.60	2.258	1.4113		
7.30	2.26		3.23		c	w.		1.42	2.010	1.4155		
7.96	2.30		3.46		d	w.						
6.88	2.29	1.97	3.00	1.16	f	w.		1.59	2.224	1.3187		
7.26	2.40	1.90	3.03	1.26	d	w.		1.42	1.978	1.3930		
7.73	2.19	1.81	3.53	1.21	d	w.		1.58	2.241	1.4184	766	
7.76	2.38		3.26		c	w.		1.54	2.186	1.4195		
7.26	2.41	1.91	3.01	1.26	d	w.		1.55	2.164	1.3961		
7.00	2.33		3.00		c-f	w.		1.38	1.934	1.4014		
7.36	2.45		3.00		d	w.		1.64	2.324	1.4171		
7.73	2.24	1.80	3.45	1.24	d	w.		1.53	2.158	1.4105	779	
7.40	2.35		3.15		d	w.						

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of				Weight of 100 grains g.
							Empty glumes	Glumes	Awn	Tip of glumes	
Mwe-swe	Myaungmya				0-7	D	w.	com.	w.	com.	
Naing-ngauchet	Myaungmya	9.61	2.85	2.14	0	D	w.	com.		l.b.	2.801
Nat-pye-hmwe Kauk Hnyin	Bassein				0	D-F	w.	l.b.		l.b.y.	
Natyewkwe	Kyaukpyu				0	C	w.	b.		l.b.	
Nga-cheik Hmwe	Tharrawaddy				0	D	b.	pur.b.		pur.b.	
Nga-pyaw-myit	Myaungmya				0-10	D	w.	l.o.	w.	l.o.	
Nga-shin-thwe I	Myaungmya	9.64	2.99	2.08	0	D	w.	l.o.		l.o.	2.409
Nga-shin-thwe II	Myaungmya	9.80	2.91	2.14	0-5	D	w.	b.	l.b.	b.	2.817
Ngwe-Maung	Akyab				0	C-D	w.	l.o.		l.b.	
Niga Lag	Mergui	9.86	2.69	2.14	0	C-F	w.	b.		l.b.	
Pauk-wa	Myaungmya				0-7	D	w.	l.o.	w.	l.o.	
Pauk-wa-gale	Myaungmya	10.09	2.87	2.10	0	C-D	w.	l.o.		b.	2.993
Pauk-wa Kauk Hnyin	Pegu				0-5	C-D	w.	l.o.	w.	l.o.	
Pyu-ban	Myaungmya				0-7	D	l.b.	b.y.	l.b.	b.	
Sapani-gale	Tharrawaddy				0-5	D	w.	l.o.	l.b.	l.b.	
Saw-ni	Myaungmya				0	F	w.	b.y.		b.y.	
Sat-tha-pu	Myaungmya				0	D	w.	com.		com.	
Sa-wa-naing	Myaungmya				0-5	C-D	w.	l.o.	w.	l.o.	
Se-ma-san	Myaungmya				0-3	D	w.	l.o.	w.	l.o.	
Shan-ma	Akyab				0	C	w.	com.		com.	
Shwebyaing	Hemzada	10.26	2.72	2.03	0	E	w.	com.		com.	2.712
Shwe-Byaing I	Sandoway				0	D-E	w.	com.		com.	
Shwe-Byaing II	Sandoway				0	C	w.	com.		com.	
Shwe-Kyu Kaung-hnyin Myaflacha-ung	Bassein				0	C-D	w.	com.		com.	
Shwelandoe	Myaungmya	10.09	3.04	2.17	5-20	C	w.	b.	l.b.	b.	3.125
Sin-doe	Myaungmya	10.28	3.12	2.23	0-20	C-F	w.	b.	l.b.	b.bl.	3.371
Wetsi Kauk-hnyin	Pegu				0	C	l.b.	b.y.		dar.b.	

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	
7.17	2.37		3.03		d	w.		1.49	2.082	1.3973	
7.26	2.23	1.85	3.26	1.21	d	w.		1.52	2.153	1.4164	769
7.46	2.27		3.29		d-f	w.		1.38	1.918	1.3899	
7.29	2.30		3.17		c	w.					
7.25	2.41		3.0		d	pur. bl.		1.47	2.012	1.3687	
7.22	2.26		3.19		d	w.					
7.07	2.35	1.83	3.01	1.28	d	w.		1.34	1.862	1.3896	773
7.08	2.34	1.97	3.03	1.19	d	w.		1.58	2.197	1.3905	780
7.45	2.37		3.14		c-d	w.		1.57	2.218	1.4127	
7.25	2.32	1.81	3.13	1.28	c-f	w.		1.47	2.094	1.4245	
7.13	2.37		3.01		d	w.		1.45	2.032	1.4014	
7.60	2.36	1.83	3.22	1.29	c-d	w.		1.62	2.322	1.4333	776
7.14	2.38		3.00		d	w.					Glumes lighter colored on veins.
7.31	2.27		3.22		c-d	w.					Glumes lighter colored on veins.
7.55	2.20		3.43		a	w.					Glumes lighter colored on veins.
7.03	2.34		3.00		c-f	t.c.					
7.07	2.28		3.10		d	w.					
7.32	2.44		3.00		c-d	w.		1.54	2.162	1.4039	Glumes lighter colored on veins.
7.05	2.35		3.00		d	w.					
7.62	2.37		3.22		c	w.					
7.54	2.22	1.81	3.40	1.23	d	w.		1.50	2.103	1.4020	775
7.32	2.16		3.39		d	w.					
7.57	2.28	1.90	3.32	1.20	c	w.		1.60	2.224	1.3900	
7.55	2.43		3.11		c-d	w.					
7.61	2.38	1.91	3.20	1.25	c	w.		1.72	2.472	1.4372	791
7.76	2.38	1.98	3.26	1.20	c-f	w.		1.88	2.622	1.3947	778
7.20	2.30		3.13		c	w.					Glumes lighter colored on veins.

S M A

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Weight of g. 100 grains	Specific gravity
							Empty glumes	Glumes	Awn	Tip of glumes	
Mweswe	Ihemzada	10.23	2.44	1.93	0	D	w.	com.		1.b.	2.121
Mwe-Swe-Kaung- Hnyin }	Bassein	10.62	2.46	1.93	0	E	w.	com.		1.b.	2.140
Myauk-Maung	Myaungmya				0	E	w.	com.		1.b.	1.119

L L.

G., S., s.

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length m.m.	Proportion between Thickness and Breadth	Shape	Colour	Abdominal white	Volume of c.c. 100 grains	Weight of 100 grains	Specific gravity	
											Proportion between wts. of unhulled and hulled grain
7.23	2.05	1.69	3.53	1.21	d	w.		1.17	1.642	1.4034	774
7.40	1.97	1.64	3.76	1.20	d	w.		1.16	1.646	1.4190	769
7.17	2.03	1.74	3.53	1.17	d	w.		1.19	1.692	1.4219	

## LONG-G

L A R

Variety	District	Unhulled grain										Specific gravity	
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Weight of 100 grains			
							Empty glumes	Glumes	Awn	Tip of glumes			
Akogyi	Myaungmya	10.70	3.71	2.30	0	F	w.	com.		l.b.	3.954	1.139	
Bagyigaung	Hemzada	10.31	3.52	2.29	0	F	l.b.	pur.b.		b.bl.	3.818	1.135	
Baw-gyi	Myaungmya	10.20	3.51	2.27	0	F	w.	com.		com.	3.408	1.156	
Baw-zye	Myaungmya				0	F	w.	com.		com.			
Bogyi	Myaungmya	10.24	3.49	2.30	0	F	w.	com.		com.	3.494	1.140	
Eikthun-magyi	Hemzada				0	F	b.	com.		pur.b.			
Eitthungyi	Bassein				0	F	w.	b.y.		l.b.y.			
Hle-w-Swe	Prome				0	F	b.	dar.b.		b.bl.			
Hleu-Swe	Hemzada	10.72	3.57	2.24	0	F	l.b.	pur.b.		b.	3.420	1.137	
Hnan-thwe	Sandoway				0	F	w.	com.		com.			
Hlay-v-Swe	Bassein				0	F	l.b.	dar.b.		b.bl.			
Kauk-hnyin byu	Pegu				0-10	A-B	w.	com.	w.	com.			
Kauk-hnyin Kauk-yaung	Tavoy				0	D	b.	dar.b.		bl.b.			
Kauknyin Ngashin	Pyapon				0	F	l.b.	b.		b.			
Kauug-nyin Bawgyi	Myaungmya				0	F	w.	com.		com.			
Kaut-wa	Myaungmya	10.39	3.65	2.35	0-10	F	l.b.	buff	l.b.	dar.b.	3.753	1.114	
Kaw-nyin (On-se)	Akyab				0	F	l.b.	com.		b.			
Kout-hnyin Witsi	Mergin	9.78	3.42	2.21	0	D-F	w.	com.		dar.b.	3.280		
Kwa-toe	Myaungmya				0-10	C-F	w.	b.y.	w.	w.			
Kya-gyi	Myaungmya				0	F	w.	b.		dar.b.			
Kya-Kyi	Pegu				0	F	l.b.	b.		dar.b.			
Kyathaung-we-Kaung Hnyin	Prome				0	F	l.b.	dar.b.		dar.b.			
Kyaw Ho	Sandoway				0	C-F	w.	y.		l.y.			
Kyigan-ma	Myaungmya				0	F	l.b.	o.		b.			
Le-Kauk-nyin	Salween	10.62	3.68	2.38	0	F	w.	com.		com.	4.104	1.173	
Laung-byan	Pegu				0	F	l.y.	b.		b.			

## RAINED.

G E .

G., L., L.

Hulled grain										Remarks		
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of c.c.	Weight of 100 grains	Specific gravity		
7.99	2.99	1.99	2.67	1.50	f	w.		2.21	3.084	1.3955	780	
7.87	2.89	2.00	2.72	1.45	f	w.		2.19	3.028	1.3826	793	
7.50	2.82	1.96	2.66	1.44	f	w.		1.94	2.706	1.3948	794	
7.35	2.72		2.70		f	w.		1.90	2.680	1.4105		
7.68	2.84	2.01	2.70	1.41	f	w.		1.98	2.732	1.3798	782	
7.14	2.90		2.46		f	w.		2.00	2.776	1.3880		
7.00	2.79		2.51		f	w.						
7.05	2.92		2.41		f	w.		1.93	2.754	1.4269		
8.00	2.83	1.99	2.83	1.42	f	w.		1.94	2.678	1.3804	783	
7.61	2.64		2.88		f	w.		1.90	2.658	1.3939		
7.40	2.83		2.61		f	w.		2.05	2.842	1.3863		
6.80	2.90		2.34		a-b	w.						
7.58	2.72		2.79		d	w.					Glumes partly com. colored.	
7.04	2.90		2.43		f	w.						
7.14	2.97		2.40		f	w.		1.99	2.780	1.3970		
7.69	2.86	2.06	2.69	1.39	f	w.		2.06	2.950	1.4320	786	
7.76	2.72		2.85		f	w.		2.04	2.882	1.4127		
7.52	2.85	1.90	2.64	1.50	d-f	w.		1.90	2.660	1.4000	811	
7.43	2.68		2.77		c-f	w.		1.8	2.536	1.3858		
7.05	2.94		2.40		f	w.					Glumes com. colored on veins.	
7.26	3.11		2.33		f	w.					Glumes com. colored on veins.	
7.05	2.86		2.47		f	w.					Glumes partly com colored.	
7.40	2.70		2.74		c-f	w.						
7.32	2.89		2.53		f	w.					Glumes lighter colored on veins.	
7.78	2.95	2.07	2.64	1.43	d-f	w.		2.31	3.255	1.4091	793	Glumes yellow colored on veins.
7.00	2.80		2.50		f	w.					Long-glumed. Glumes com. colored on veins.	

Variety	District	Unhulled grain										
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of				Weight of 100 grains g.	
							Imper. glumes	Glumes	Awn	Tip of glumes		
Magyi-gaung-nyin	Myaungmya				0	F	w.	b.		b.		
Miginth-wag	Pegu				0	F	l.b.	b.		dar.b.		
Mwesokkyi	Hemzada				0	A	w.	com.		dar.b.		
Nga-cheik	Myaungmya				0	B	l.b.	dar.b.		b.bl.		
Ngapyagyi	Hemzada				0	F	l.b.	buff		dar.b.		
Ngasein	Pyapon				0	B	w.	com.		com.		
Nga-shin Thwe	Pagu	10.00	3.50	2.29	0	F	w.	b.	pur.b.	3.455	1.103	
Nga-yan-pa Naw	Myaungmya				1-10	F	l.b.	buff	l.b.	dar.b.		
Pauk-kyait	Myaungmya				0	F	w.	com.		com.		
PaukLwingyi	Toungoo				0	F	l.b.	b.		b.		
Pauk-wagyi	Myaungmya				0	F	w.	b.y.		l.b.y.		
Pya-gyi	Myaungmya				0-6	F	l.b.	buff	l.b.	dar.b.		
Pya-gyi-gaung	Myaungmya				0	F	l.b.	b.		b.		
Pyagyi-taung I	Myaungmya				0	F	w.	b.		b.		
Pya-gyi-taung II	Myaungmya	10.05	3.76	2.30	0-5	F	l.b.	b.	b.	l.b.	3.774	1.140
Shwe Hnan Paung	Pegu	10.89	3.74	2.30	0	F	w.	com.	com.	4.345	1.138	
Sin-kwa-Byu	Thaton				0	A	w.	com.		com.		
Sinkwa	Hemzada				0	F	w.	b.		l.b.		
Sin-Kwa Ni	Thaton				0	B	l.b.	b.		b.		
Sondal paddy	Sandoway				0	F	w.	l.b.y.		com.		
Thouhlo Koung Huyin }	Pyapon				0	F	w.	l.y.		l.y.		
Wetse	Sandoway				0	F	w.	com.		com.		
Wet-se-Kaung Hnyin	Tharrawaddy				0	F	w.	com.		b.		
Wet-si	Myaungmya				0-10	F	l.b.	b.	l.b.	b.		
Wet-si Kaung Knyin	Prome				0	F	w.	com.		b.		
Ye-panet	Pegu				0	B	l.b.	dar.b.		dar.b.		
Ze gwet Ma	Tharrawaddy				0	F	w.	com.		dar.b.		

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	
7.31	2.89		2.53		f	w.					Glumes com. colored on veins.
7.40	2.90		2.55		f	w.					
6.61	3.02		2.19		a	w.					Glumes spotted with b. color.
6.80	2.98		2.28		b	pur. bl.					Glumes lighter colored on veins.
7.39	3.00		2.46		f	w.		1.90	2.668	1.4042	Glumes partly com. colored.
7.13	2.85		2.50		b	w.					
7.37	2.70	1.93	2.73	1.40	f	w.		1.91	2.693	1.4099	779
7.67	2.83		2.71		f	w.		2.14	2.986	1.3953	Glumes lighter colored on veins.
7.10	2.80		2.54		f	w.					Glumes striped with light b. color.
7.21	2.93		2.46		f	w.					Glumes com. colored on veins.
7.03	2.78		2.53		f	w.					Glumes lighter colored on veins.
7.58	2.86		2.65		f	w.		2.14	2.978	1.3916	Glumes lighter colored on veins.
6.95	2.86		2.43		f	w.					Glumes com. colored on veins.
7.16	2.85		2.51		f	w.					Glumes com. colored on veins.
7.41	2.97	2.01	2.49	1.48	f	w.		2.13	2.966	1.3925	786
7.99	3.03	2.00	2.64	1.52	f	w.		2.40	3.400	1.4167	783
6.76	3.08		2.19		a	w.					Glumes oft. spotted with b. color.
6.90	2.83		2.44		f	w.					Glumes com. colored on veins.
6.90	2.90		2.38		b	w.		2.18	3.046	1.3972	Glumes l.y. colored on veins.
7.83	2.73		2.87		f	w.					
7.40	2.86		2.59		f	w.		1.88	2.604	1.3851	
7.50	2.86		2.62		f	w.					Glumes striped with b. color.
7.55	2.89		2.61		f	w.		2.21	3.92	1.3991	
7.50	2.87		2.61		f	w.		2.20	3.056	1.3891	
7.50	2.90		2.59		f	w.		2.18	3.048	1.3982	
6.62	3.02		2.19		b	pur. bl.					Glumes lighter colored on veins.
7.91	2.85		2.78		f	w.		2.11	2.976	1.4104	

## MED

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of				Weight of 100 grains g.
Aik-thun	Bassein				0-15	C	l.b.	b.y.	b.	b.bl.	
Bankauk	Toungoo				0	C-F	w.	com.		com.	
Baw-gout	Myaungmya	9.20	3.18	2.14	0	A-C	w.	com.			2.525 1.136
Baw-gyi	Myaungmya				0	F	w.	y.		com.	
Begyagi	Henzada	9.10	3.25	2.20	0	B-F	w.	b.y.		b.y.	3.092 1.141
Be-lat-ngacheik	Bassein	8.89	3.47	2.04	0	B	b.	dar.b.		bl.	
Cheik-kyi	Sandoway				0	F	w.	com.		com.	
Coconut oil	Myaungmya				0-10	F	w.	com.	w.	com.	
Doe-gale	Myaungmya				0	A	w.	b.		dar.b.	
Fike-thon	Myaungmya	10.10	3.40	2.28	0	F	w.	com.		com.	1.098
Hlay-w-Swe	Myaungmya				0	B	b.	dar.b.		b.bl.	
Hnga-Pyaw-Nyun	Pyapon				0	D-E	w.	com.		com.	
Inbaw-gyi	Prome				0	A-B	w.	b.y.		l.b.y.	
In-boak	Sandoway				0	A-F	w.	com.		com.	
Jaungya	Salween				0	A-B	w.	y.b.		l.b.	
Ka Baing	Myaungmya				0	D	w.	b.		b.	
Kaub-hnyin Nga- cheib	Tharrawaddy				0	B	l.b.	dar.b.		bl.b.	
Kaub-hnyin-Nigyi	Tavoy				0-10	D	l.b.	b.	l.b.	b.	
Kauk-hnyin Kyet- tuywe manaing	Bassein	9.65	3.28	2.13	0	F	w.	y.		l.y.	3.042 1.121
Kauknyin-ngacheik	Pyapon				0	B	l.b.	dar.b.		dar.b.	
Kauk-hnyin Ngacheik	Pegu				0	B	l.b.	dar.b.		dar.b.	
Kauk-hnyin Ngacheik	Toungoo				0	A-B	l.b.	dar.b.		dar.b.	
Kauk-hnyin Pyaungyaw	Tavoy				0-20	D	l.b.	b.	l.b.	b.	
Kauk-kyi	Myaungmya				0	F	l.b.	b.		b.	
Kauk-nyin Ngacheik	Salween				0	A-B	l.b.	pur.b.		dar.b.	
Kaung Hnyin Byu	Pyapon				0-10	D	w.	com.	w.	com.	
Kaung-nyin Dae-we	Myaungmya				0	A-B	w.	l.o.		l.o.	
Kaung hnyin	Pyapon				0	B	l.b.	b bl.		b.bl.	

I U M .

G., L., M.

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between Thickness and Breadth	Shape	Colour	Abdominal white	Volume of c.c. 100 grains	Weight of 100 grains	Specific gravity	
7.15	2.45		2.92		c	w.		1.43	2.024	1.4154	
7.11	2.49		2.86		c	w.		1.65	2.274	1.3782	
6.62	2.53	1.88	2.62	1.35	a-c	w.		1.41	1.992	1.4128	789
7.10	2.68		2.65		c-f	w.		1.69	2.378	1.4071	
6.78	2.66	1.95	2.55	1.36	b-f	w.		1.77	2.430	1.3729	786
6.66	2.66	1.77	2.50	1.50	a-f	pur. bl.		1.30	1.774	1.3646	
6.86	2.79		2.46		d-f	pur. b <sup>l</sup> .		1.73	2.422	1.4000	
7.26	2.54		2.86		f	w.		1.62	2.264	1.3975	
6.00	2.75		2.18		d	w.		1.62	2.248	1.3877	
6.75	2.73	2.02	2.47	1.35	f	w.					Glumes com. colored on veins.
6.58	2.82		2.33		a-b	pur. bl.		1.76	2.416	1.3727	
7.05	2.40		2.94		c-d	w.		1.50	2.082	1.3880	
6.41	2.62		2.45		a-b	w.		1.66	2.294	1.3819	
6.48	2.77		2.34		a-f	pur. bl.		1.52	2.104	1.3842	
6.50	2.77		2.35		a-b	t.c.					Glumes lighter colored on veins.
7.15	2.62		2.73		c	t.c.		1.86	2.546	1.3688	
6.78	2.72		2.49		b	pur. bl.		1.71	2.364	1.3825	
6.95	2.70		2.57		d	w.		1.72	2.416	1.4047	
7.19	2.65	1.84	2.71	1.44	f	w.		1.68	2.370	1.4107	779
6.52	2.78		2.35		b	pur. bl.		1.68	2.310	1.3750	
6.40	2.77		2.31		a-b	pur. bl.		1.64	2.260	1.3780	
6.17	2.84		2.17		a-b	pur. bl.		1.69	2.376	1.4059	
7.20	2.60		2.77		d	w.		1.87	2.590	1.3850	
6.52	2.70		2.41		f	w.		1.59	2.218	1.3950	
6.55	2.50		2.62		a-b	pur. bl.					Glumes lighter colored on veins.
7.34	2.45		2.99		d	w.					
6.16	2.65		2.32		a-b	w.		1.60	2.208	1.3800	
6.60	2.84		2.32		b	pur. bl.					Glumes oft. pale gr. colored on veins.

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of			Weight of 100 grains	Specific gravity
							Empty glumes	Glumes	Awn		
Kaung-nyin eike-thu	Myaungmya				0	F	l.b.	b.y.		b.	
Kaung-nyin Kye-pyat-gyi	Myaungmya				0-10	F	w.	y.b.	w.	y.b.	
Kaung-nyin-panku	Myaungmya				0	A-B	w.	l.b.y.		l.b.y.	
Kauug-nyin Saba	Myaungmya	9.89	3.35	2.19	0	B-F	l.b.	b.y.		b.	3.284
Kaung-nyin Sanni	Myaungmya				0	A	w.	o.		b.	
Kouknyin Jaw Nat	Pyapon				0	C	w.	b.y.		b.y.	
Kout Hnyin-chout	Mergui	9.59	3.32	2.25	0	E-F	w.	com.	com.		2.843
Kout Kauk-hnyin	Pegu				0	C	w.	b.		dar.b.	
Kyaw-sin	Myaungmya				0	F	w.	b.y.		b.y.	
Kyaw-zin	Bassein				0	B	w.	l.b.y.		l.b.y.	
Kyeb-tu-ywe-Mauai	Myaungmya				0	C	w.	y.		l.y.	
Kyetpagyi	Toungoo				0	C-F	w.	com.		com.	
Kyikanma	Hemzada	9.25	3.25	2.20	0	D	l.b.	dar.b.	dar.b.		2.935
Kyikanma	Bassein				0	D	l.b.	b.bl.		bl.	
Kywet-thwa	Myaungmya	8.93	2.95	2.05	0	C	w.	y.		b.y.	
Lei-ngacheik	Myaungmya				0	B-D	l.b.	dar.b.		dar.b.	
Lei-ngacheik	Bassein				0	B	l.b.	l.pur. b.		dar.b.	
Leik-kale I	Myaungmya				0	C-F	w.	y.		y.	
Leik-ka-le II	Myaungmya				0	A	w.	b.		l.b.	
Lin-ma-no	Myaungmya	8.54	3.38	2.20	0	A	w.	y.b.	dar.b.		2.945
Mein Ma Illa	Pegu	9.95	3.48	2.29	0-10	B-C	w.	com.	w.	com.	3.571
Mi-kale	Myaungmya				0	C	w.	y.		y.	
Nga-cheik	Myaungmya				0	B	l.b.	dar.b.		b.bl.	
Nga-cheik	Hemzada				0	B	l.b.	dar.b.		b.bl.	
Nga-cheik	Pegu				0	B	l.b.	dar.b.		b.bl.	
Nga-cheikyin	Hemzada				0	A	l.b.	l.pur. b.		pur.bl.	
Nga-kyeik	Sandoway				0	D	w.	com.		com.	
Nga Pya-tuyi	Maubin				0	F	l.b.	l.b.		b.pur.	
Ngashin-thwe	Bassein				0	C-F	w.	b.		b.	
Nga-shin-thwe	Myaungmya				0	A	w.	b.y.		b.y.	

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Proportion between Breadth and Length Thickness and Breadth		Shape	Colour	Abdominal white	Volume of 100 grains c. c.	Weight of 100 grains g.	Specific gravity	
7.33	2.51	2.92			a-f	w.					Glumes lighter colored on veins.
7.40	2.54	2.91			f	w.					Glumes lighter colored on veins.
6.55	2.65	2.47			a-b	w.		1.40	1.938	1.3843	
6.90	2.52	1.99	2.74	1.27	b-f	w.		1.83	2.561	1.4020	780
6.67	2.86	2.33			a	t.c.					Glumes lighter colored on veins. Glumes lighter colored on veins.
7.33	2.55	2.87			c	w.					
6.88	2.61	1.91	2.64	1.37	d-f	pur. bl.		1.62	2.286	1.4111	804
7.05	2.65	2.66			c	w.		1.81	2.540	1.4033	
6.76	2.75	2.46			f	w.					Glumes lighter colored on veins.
6.48	2.93	2.21			b	w.					Glumes lighter colored on veins.
7.28	2.53	2.88			c	w.					Glumes lighter colored on veins.
7.07	2.51	2.82			c	w.					Glumes lighter colored on veins.
6.85	2.57	1.96	2.67	1.31	a-d	w.		1.62	2.247	1.3870	766
7.00	2.60	2.69			a-d	w.					Glumes lighter colored on veins.
6.38	2.40	1.79	2.66	1.32	c	w.		1.27	1.780	1.4016	783
6.55	2.85	2.30			b-d	pur. bl.					Glumes com. colored on veins.
6.60	2.78	2.37			b	pur. bl.					Glumes lighter colored on veins.
7.30	2.59	2.82			c-f	w.					Glumes lighter colored on veins.
6.05	2.90	2.09			a	w.		1.58	2.176	1.3772	
6.50	2.82	1.90	2.30	1.48	a	t.c.		16.4	2.268	1.3829	770
6.78	2.80	19.6	2.42	1.43	b-c	w.			2.786		780
7.20	2.50	2.88			c	w.					
6.25	2.80	2.23			b	pur. bl.					Glumes lighter colored on veins.
6.36	2.82	2.26			b	pur. bl.					Glumes lighter colored on veins.
7.00	2.67	2.62			a-b	pur. bl.					Glumes lighter colored on veins.
6.45	2.65	2.43			a	pur. bl.					Glumes portly com. colored.
6.76	2.80	2.41			d	pur. bl.					Abd. half of the hulled grain l.b. colored.
7.20	2.60	2.77			f	w.					Glumes pertly com. colored.
6.78	2.77	2.45			c-f	w.					
6.46	2.70	2.39			a	w.					Glumes lighter colored on veins.

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	Thickness m.m.	Length of awn m.m.	Shape	Colour of				Weight of 100 grains g.
							Empty glumes	Glumes	Awn	Tip of glumes	
Ngashin-thwe	Thaton				0	B	l.b.	b		b.	
Nget Pyaw Myit	Myaungmya				0	F	w.	com.		com.	
Nyan-yo	Bassein				0	F	w.	y.		y.	
Ou-si	Myaungmya				0	A	w.	l.o.		l.o.	
Paubwa-magy	Tharrawaddy	9.10	3.25	2.21	0	F	w.	b.y.		l.b.y.	2.775 1.142
Paub-wa-yin	Tharrawaddy				0-23	B	w.	b.y.	w.	l.b.y.	
Paub-wa I	Myaungmya				0	F	w.	b.y.		b.y.	
Pauk-wa II	Myaungmya	9.60	3.43	2.16	0	F	w.	b.y.		l.b.y.	3.120 1.131
Pauk-wa	Sandoway				0	B	w.	y.		y.	
Pauk-wa-yin	Prome				0	C-F	w.	l.y.b.		com.	
Pegu	Myaungmya				0	F	w.	l.o.		l.o.	
Poukwa	Kyaukpyu	8.60	3.21	2.10	0-16	B	w.	y.	w.	y.	2.719 1.166
Pya ye-san	Myaungmya	9.59	3.29	2.19	0	B-C	w.	y.		l.y.	3.106 1.144
Pye-gyichoke	Myaungmya	9.38	3.36	2.23	0-10	A-C	w.	l.o.	w.	com.	3.327 1.148
Pye-gyi	Sandoway				0	B	w.	y.		y.	
Pyi-gyi-gyoke	Myaungmya				0	F	w.	l.o.		l.o.	
Sa-ba-dae-we	Myaungmya				0	B	w.	l.o.		l.o.	
Se-ma-san	Myaungmya				0	A-F	w.	l.o.		l.o.	
Shwe Hlando	Pegu				0-20	C	l.b.	b.	l.b.	dar.b.	
Shwelando	Myaungmya				0-15	C-F	l.b.	b.	l.b.	dar.b.	
Shwe-wa	Myaungmya				0	F	l.b.	b.		b.	
Sinkwa	Pegu				0	A-B	l.b.	b.		l.b.	
Smai-gyi	Myaungmya				0	B	w.	com.		l.b.	
Tau Ba	Thaton				0-15	C-F	w.	l.y.	w.	l.y.	
Taung-Daik-Pan	Bassein				0	C	w.	com.		com.	
Taung-yo-Kaung Hnyin	Tharrawaddy				0	D	w.	com.		com.	
Taung-yo- ngacheik I	Myaungmya				0	B	w.	com.		dar.b.	
Taung-yo- ngacheik II	Myauugmya				0	B	w.	com.		dar.b.	
Thautwen	Kyaukpyu				0	B	l.b.	b.bl.		l.bl.	
Thouhau-byoot	Kyaukpyu				0	B	w.	buff		buff	

Hulled grain										Remarks	
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between	Shape	Colour	Abdominal white	Volume of c.c.	Weight of g. 100 grains		
										Proportion between wts. of unhulled and hulled grain	
6.58	2.64			2.49	b	w.					
7.37	2.58			2.86	f	w.				Glumes streaked with l.b. color.	
6.58	2.58			2.55	f	w.					
6.42	2.66			2.41	a	w.				Glumes lighter color- ed on veins.	
6.77	2.65	2.02		2.55	1.31	f	w.	1.55	2.178	1.4052	Glumes lighter co'ord on veins.
6.50	2.90			2.24	b	w.				Glumes lighter color- ed on veins.	
6.68	2.80			2.39	f	w.				Glumes lighter color- ed on veins.	
6.82	2.70	1.85		2.53	1.46	f	w.	1.72	2.410	1.4012	Glumes lighter color- ed on veins.
6.42	2.60			2.47	b	w.				Glumes lighter color- ed on veins.	
7.15	2.70			2.65	c-f	w.				Glumes lighter color- ed on veins.	
7.00	2.60			2.69	f	w.					
6.44	2.55	1.83		2.53	1.39	b	w.	1.54	2.132	1.3844	Glumes lighter color- ed on veins.
7.19	2.69	1.90		2.67	1.42	b-c	w.	1.72	2.438	1.4174	Glumes lighter color- ed on veins.
7.06	2.67	1.99		2.64	1.34	a-c	w.	1.86	2.582	1.3882	Glumes lighter color- ed on veins.
6.40	2.60			2.46	b	w.				Glumes lighter color- ed on veins.	
6.60	2.75			2.40	f	w.				Glumes lighter color- ed on veins.	
6.73	2.75			2.45	b	w.				Glumes lighter color- ed on veins.	
6.95	2.65			2.62	a-f	w.				Glumes lighter color- ed on veins.	
7.10	2.43			2.92	c	w.					
7.10	2.40			2.96	c-f	w.					
6.55	2.95			2.22	f	w.					
6.01	2.70			2.23	b	w.				Glumes lighter color- ed veins.	
6.55	2.55			2.57	b	pur. bl.		1.56	2.196	1.4077	
7.20	2.61			2.76	c-f	w.				Glumes lighter color- ed on veins.	
7.35	2.50			2.94	c	w.		1.65	2.326	1.4097	
6.73	2.62			2.57	d	pur. bl.		1.55	2.170	1.4000	
6.64	2.58			2.57	b	pur. bl.		1.50	2.056	1.3707	
6.63	2.70			2.46	b	pur. bl.					
5.87	2.72			2.16	a	w.		1.39	1.946	1.4000	Glumes lighter color- ed on veins.
6.52	2.77			2.35	b	pur. bl.				Glumes lighter color- ed on veins.	

Variety	District	Unhulled grain									
		Length m.m.	Breadth m.m.	'Thickness m.m.	Length of awn m.m.	Shape	Colour of				Weight of 100 grains
							Empty glumes	Glumes	Awn	Tip of glumes	
Tourg-yo-Ngacheik	Pyapon				0	B	w.	buff		buff	
Uga-Pya-gyi	Prome				0	F	l.b.	pur.b.		dar.b.	
Unbonk	N. Arakan	9.18	2.94	2.13	0-5	D	w.	com.	w.	l.b.	
Wun-gauk	Myaungmya				0	A	w.	com.		dar.b.	
Ywetaung	Hemzada				0	F	l.b.	dar.b.		dar.b.	
Zaw-nyun	Myaungmya				0	B	w.	l(buff)		com.	

## S M A

Hnget-Pyaw Myit	Bassein				0	A-C	w.	com.		dar.b.	
Khwanwa	Akyab				0	B	w.	o.		o.	
Kunwa	Myaungmya				0	B	w.	l. buff		l. buff	
Kyathaung-bwe	Hemzada	8.80	3.28	2.22	0	B	l.b.	pur.b.		pur.b.	
Louhtat	Tavoy				0	F	w.	com.		com.	
Nget-pyaw Nyum	Myaungmya				0	C	w.	com.		b.bl.	

## Hulled grain

Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length Thickness and Breadth	Proportion between Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains g.	Specific gravity	Proportion between wts. of unhulled and hulled grain	Remarks
6.80	2.85		2.39	b	pur. bl.		1.59	2.196	1.3811		Glumes lighter colored on veins.
7.00	2.74		2.55	f	w.		1.73	2.428	1.4035		Glumes com. colored on veins.
6.85	2.45	1.85	2.80	1.32	d	pur. bl.	1.50	2.132	1.4213		
6.50	3.05		2.13	a	w.		1.83	2.568	1.4033		Glumes oft. spotted with b. color.
6.76	2.50		2.70	f	w.		1.48	2.008	1.3568		Glumes partly com. colored.
6.18	2.70		2.29	b	w.		1.60	2.226	1.3913		Glumes lighter colored on veins.

L L .

6.52	2.30		2.83	a-c	w.		1.48	2.096	1.4162		Glumes spotted with b. color.
6.00	2.45		2.45	b	w.		1.50	2.082	1.3880		Glumes w. tinted.
6.00	2.50		2.40	a-b	w.		1.41	1.958	1.3887		
6.04	2.48	1.96	2.44	1.27	a-f	w.	1.57	2.194	1.3975		Glumes partly light b. colored.
6.48	2.30		2.82	f	w.		1.38	1.940	1.4058		
6.47	2.30		2.81	c	w.		1.36	1.888	1.3882		

**SHORT-G****L A R**

Variety	District	Unhulled grain										Specific gravity
		Length		Breadth		Thickness		Length of awn		Shape		
		m.m.	m.m.	m.m.	m.m.	m.m.	m.m.	m.m.	m.m.	Empty glumes	Glumes	Awn
Baide	N. Arakan	8.35	3.95	2.50	0	F	l.b.	com.		l.b.		
Leikkyi	Hemzada	8.19	3.80	2.36	0	A	l.b.	b.		dar.b.		1.127

**M E D**

Kyigau-gallung-nyin	Myaungmya	7.50	3.50	2.40	0	A-B	l.b.	b.bl.		b.bl.	2.870	1.158
Kyigaw Ma	Pyapon	8.09	3.50	2.27	0	A	l.b.	b.bl.		b.bl.	2.762	1.150
Leik-kalé	Hemzada	8.14	3.80	2.28	0	A	w.	com.		com.	3.214	1.116
Pannyo Kauk-hnyin	Pegu				0	A	l.b.	b.bl.		b.bl.		

**S M A**

Ja-loke-gyi	Myaungmya				0	A-B	w.	b.		dar.b.	2.910	
Kyauk Kauk-hnyin	Thaton	5.80	3.84	2.30	0	F	w.	b.		b.	2.002	1.089
Leik-kale	Myaungmya	5.75	3.70	2.30	0	F	w.	b.		b.	1.820	
Sapamin Myo	Pegu	6.24	3.95	2.32	0	F	w.	b.		dar.b.	2.157	1.117

## RAINED.

G E .

G., S., L., M. &amp; S.

Hulled grain											Remarks
Length m.m.	Breadth m.m.	Thickness m.m.	Breadth and Length	Proportion between Thickness and Breadth	Shape	Colour	Abdominal white	Volume of 100 grains c.c.	Weight of 100 grains	Specific gravity	
6.78	3.40	2.22	1.99	1.53	f	w.		2.11	2.968	1.4066	
5.92	3.05	1.94	1.94	1.57	a	w.		1.84	2.574	1.3989	Glumes partly com. colored.

I U M .

5.92	2.98	1.94	1.99	1.54	a-b	w.		1.58	2.216	1.4025	772	Glumes b. colored on veins.
5.74	2.97	1.94	1.93	1.53	a	w.		1.55	2.154	1.3897	780	Glumes b. colored on veins.
5.53	3.13	2.02	1.77	1.55	a	w.		2.530			787	
5.78	2.93		1.97		a	w.						Glumes b. colored on veins.

L L .

5.65	2.85		1.98		a-b	w.		1.59	2.194	1.3799	754	Glumes com. colored on veins.
4.16	3.28	1.93	1.27	1.70	f	w.		1.06	1.448	1.3660	723	Glumes lighter colo- red on veins
4.06	3.16	1.92	1.28	1.65	f	w.		1.270			698	Glumes lighter color- ed on veins.
4.16	3.29	1.90	1.26	1.73	f	w.		1.08	1.516	1.4037	703	Glumes lighter color- ed on veins.

## EXPLANATION OF PLATES.

## PLATES V.

Fig. 1. *Oryza granulata*, Nees. (Specimen in Kew Herbarium).

Fig. 2. *Oryza granulata*, Nees. (Specimen in Kew Herbarium).

## PLATE VI.

Fig. 3. *Oryza latifolia*, Desv. (Specimen in Kew Herbarium).

Fig. 4. *Oryza coarctata*, Roxb., from the Delta of the Ganges, India. (Specimen in Kew Herbarium).

## PLATE VII.

Fig. 5. *Oryza granulata*, Nees. (Specimen in Kew Herbarium).

Fig. 6. An Indian salt rice and three Japanese common rices irrigated with an artificial sea water from July 15 to July 27. (Photo. in July 30, 1908).

## PLATE VIII.

Fig. 7. *Oryza coarctata*, Roxb., from Sangor Islands, India. (Specimen in Indian Museum).

Fig. 8. *Oryza coarctata*, Griffith, from Khalen, Burma. (Specimen in Indian Museum).

Fig. 9. *Oryza coarctata*, Roxb., from Sunderbuns, India. (Specimen in Indian Museum).

Fig. 10. A collection of grains of *Oryza coarctata*. (Specimens in Indian Museum).

Fig. 11. *Oryza latifolia*, Desv. from Palaw, Mergui, Burma. (Specimen in Indian Museum).

Fig. 12. Wild rice (*O. sativa*) naturally growing in Klon Rangsit, Siam.

Fig. 13. Specimen of wild rice from Klon Rangsit, Siam. (Whole plant).

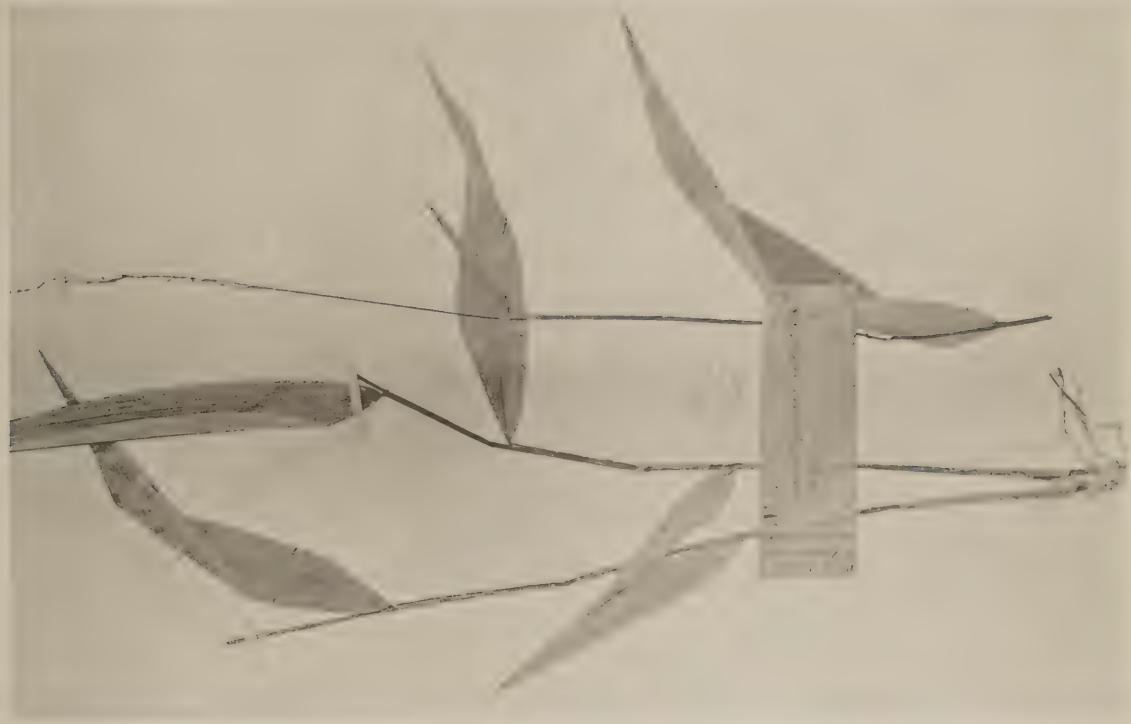
Fig. 14. A portion of the specimen of wild rice from Klon Rangsit, Siam.

Fig. 15. A giant rice, cultivated in Ayuthia, Siam, and an ordinary Japanese rice, Shinriki.

Fig. 16. A dwarf rice, Daikoku.

Fig. 17. Six main types of shape of Burman rice.

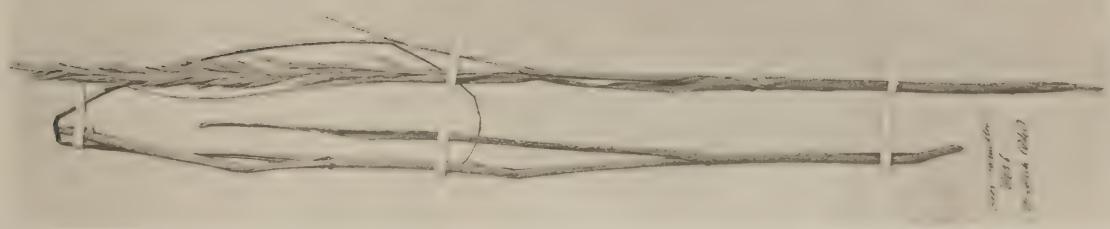
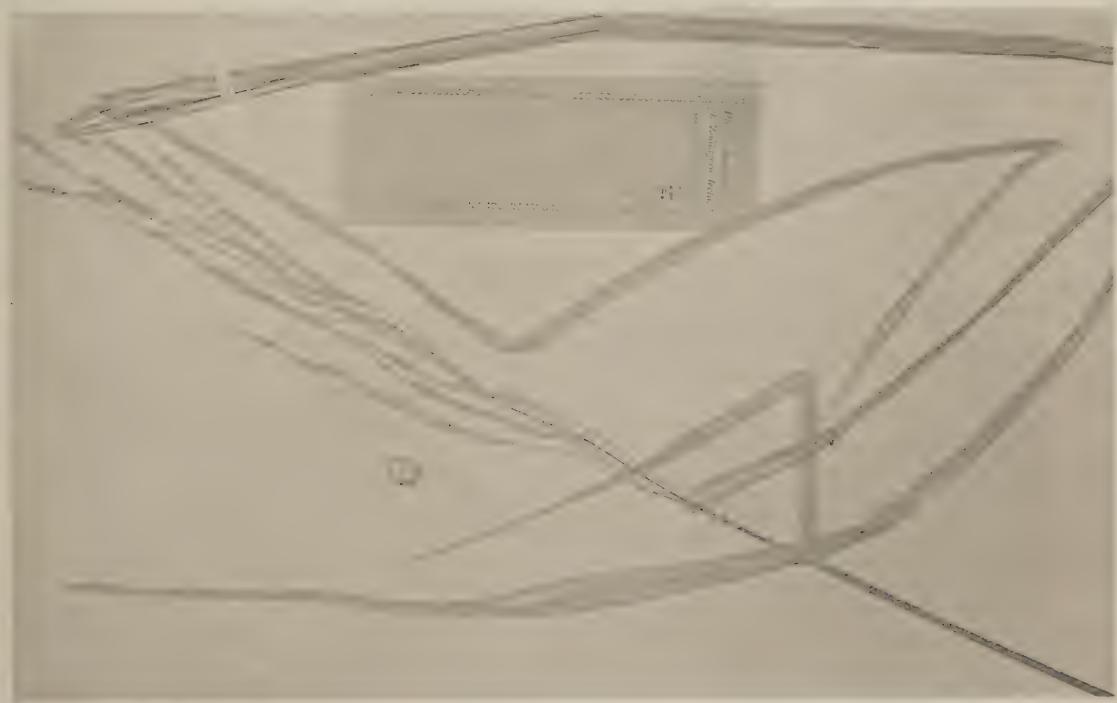
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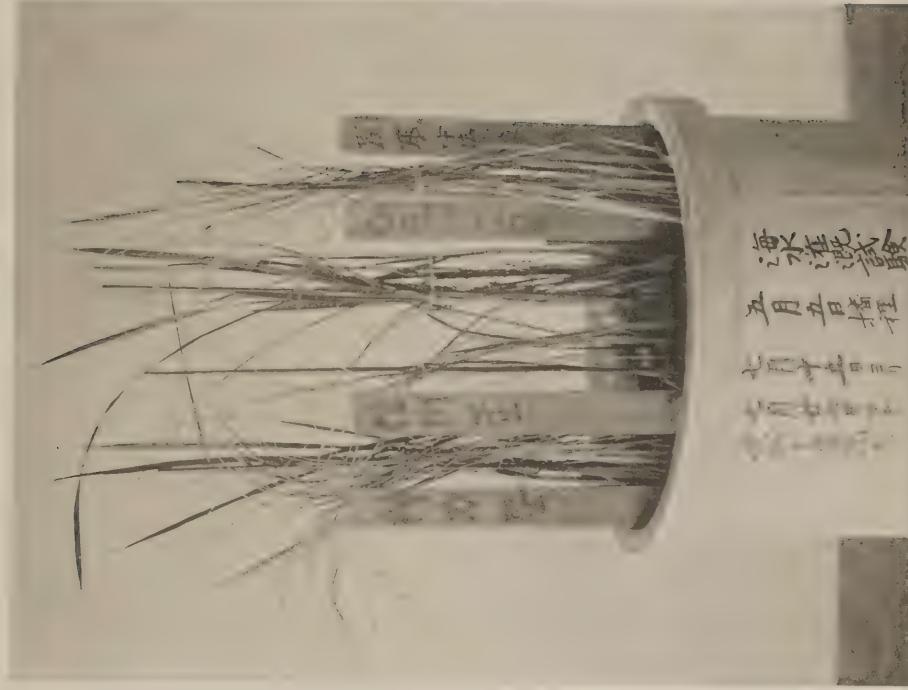


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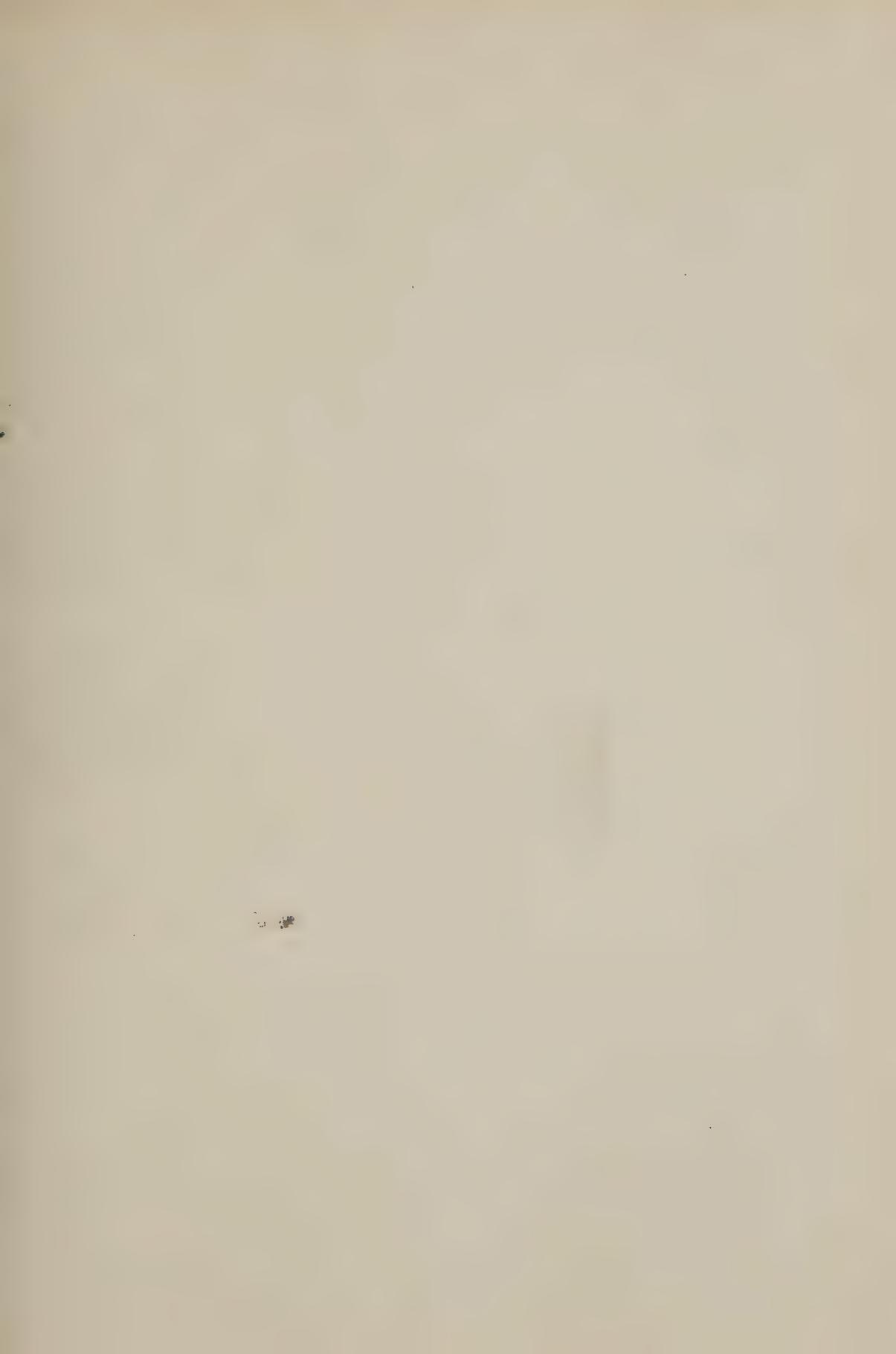
*Carex crenata* Nutt.  
f. *Acuminata* (C. M.)

var. *acuminata*  
Nutt.  
(C. M.)









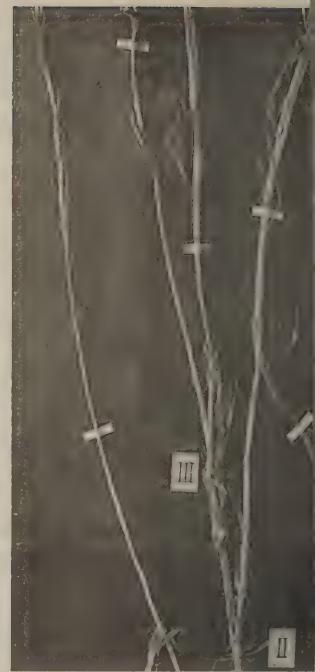
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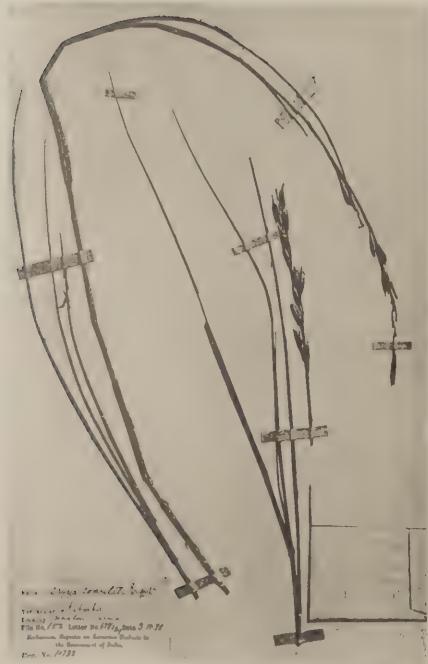
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14.



8.



11.



A.	<i>O. Coarcta</i>
B.	"
C.	"
D.	"
E.	"



12.



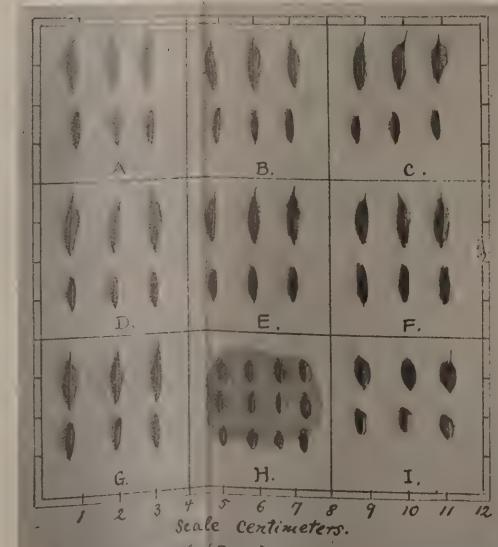
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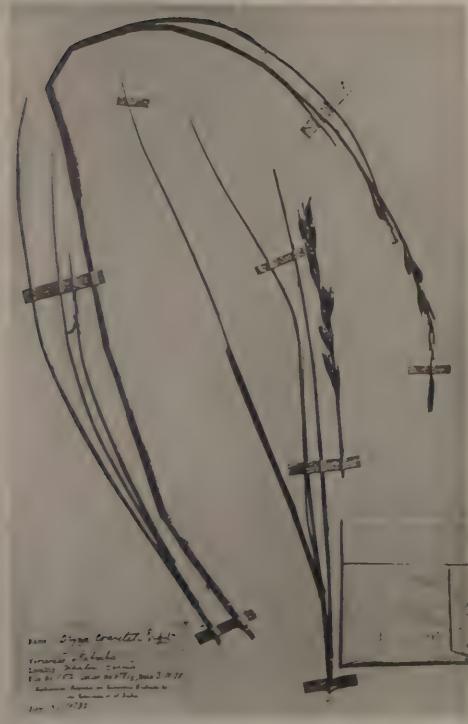
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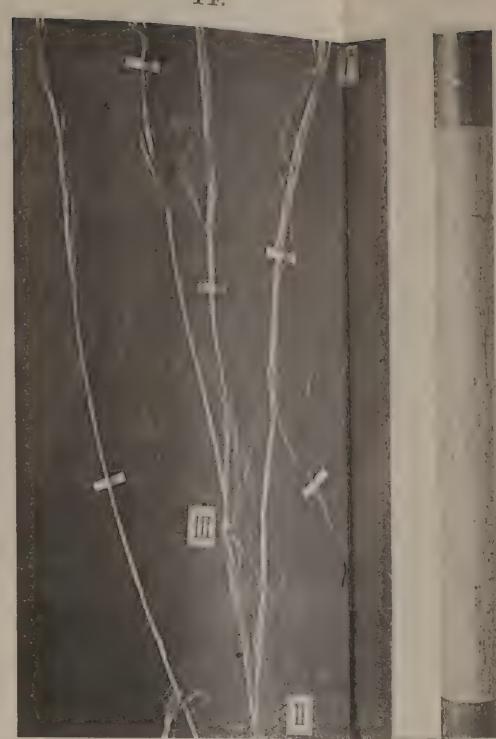
A.	<i>O. Coarctata</i> (Burm.)	F. " " (Phalon)
B.	" ( <i>Sanderianus</i> )	G. " " ( <i>Tennaserim</i> )
C.	" ( <i>Bonkay</i> )	H. <i>O. latifolia</i> (Akyat)
D.	" ( <i>Beckergunjye</i> )	I. <i>O. sativa</i> (Hadagall)
E.	" ( <i>Moulmein Tennaserium</i> )	



9



14.



15



13.



12.



H. Oye, imp.



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All communications relating to this Journal should be addressed to the Director of the College of Agriculture.

MAR 23 1917

## Some Studies on the Germination of the Seed of *Oryza Sativa*.

By

**Isaburo Nagai.**

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With Plate IX and two Text-Figures.

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The present paper deals with some of the experimental results by which it is attempted to verify and to give some further data on the physiology of germination of the seed in Gramineae. Only a few phases, however, that are involved in the course of germination, are touched upon, and the experiments are conducted almost exclusively with the seed of *Oryza sativa* L., but for some phases *Zea Mays* L. and other seeds are studied. The subject will be treated in the following sections.

- (1) Rôle of the selective-permeable septum of the seed covering in the viability of the seed.
- (2) The seat of the selective-permeable septum in the seed covering.
- (3) Rôle of oxygen in germination.
- (4) Effect of  $H$  and  $OH$  ions in germination.
- (5) Influence of extremes of temperature on the germinative power.

### I. Rôle of the Selective-Permeable Septum of the Seed Covering in the Viability of the Seed.

According to the manner in which water is chiefly taken up, the seeds of higher plants may be classified into two types, namely, one in which water is absorbed through the seed covering which possesses the power of selective permeability but no significant water conduction by the micropylar opening; the other, on the other hand, in which water is absorbed not only by diffusion through the seed covering but also directly by the opening (micropyle) into the seed.

The seed therefore, which belongs to the first type would be safely guarded against the action of toxic solutions when subjected to them. On the other hand, the seed of the second type, such as that of Leguminosae, would be less resistant, for there is a micropylar opening which conducts the toxic solution into the seed, even though the seed is wrapped up in the "semi-permeable" septum, just as in the former type.

It was BROWN (1907, 1909) who first showed the presence of a "semi-permeable" septum in the seed covering of *Hordeum vulgare*, var. *coeruleascens*, of *Triticum*, of *Avena* and of *Secale*. This fact is extended to *Oryza* (VALETON 1907), *Triticum* (SCHROEDER 1911), *Avena sativa* (ATWOOD 1914), *Xanthium glabratum*, *Helianthus annuus*, *Vicia Faba*, scarlet runner, lima bean, *Alisma Plantago-aquatica*, peach, apple (SHULL 1913), and sugar beet (TJEBBES 1912).

The seed covering is reported to exclude the passage of the following : sodium fluoride, potassium chloride, sodium chloride, potassium nitrate, potassium carbonate, sodium carbonate, barium chloride, sodium sulphate, magnesium sulphate, silver nitrate, cobalt chloride, seignette salt, cane sugar (wheat : SCHROEDER 1911), sulphuric acid, hydrochloric acid, copper sulphate, ferrous sulphate, potassium chromate, silver nitrate, potassium ferrocyanide, sodium hydroxide (0.5%), sodium chloride, sodium nitrate, potassium chloride, potassium nitrate, tartaric acid, cane sugar, dextrine, ammonium chloride, sodium acetate, (barley: BROWN 1907, 1909), sodium chloride (wild oats: ATWOOD 1914), sodium chloride, copper sulphate, potassium chromate, sodium thiosulphate, glycerol, sucrose, fructose, glucose, lactose, hydrochloric acid, tartaric acid, and citric acid (*Xanthium* : SHULL 1913); but to give entrance to the following, slowly or rapidly : sublimate, iodine, methyl and ethyl alcohol, ethyl ether acetone, acetonitril, chloroform, osmic acid (wheat : SCHROEDER 1911), ammonia, cadmium chloride, cadmium sulphate, glycocollic acid,\* lactic acid,† glycerol, glycerine, urea, ethylene glycol, acetic acid, trichloracetic acid, ethyl alcohol, ethyl acetate, aldehyde, acetone, ethylic acetate (barley : BROWN 1909), ammonium nitrate, silver nitrate, sodium nitrate, potassium nitrate, ferrous sulphate, potassium chloride, mercuric chloride, iodine in alcohols, ether, alkalies, sulphuric acid, nitric acid, acetic acid, and lactic acid (*Xanthium* : SHULL 1913).

\* Excluded until the lapse of 48 hours.

† Excluded until the lapse of 2-9 hours.

The works of NOBBE (1876), PRILLIEUX (1878), VAN TIEGHEM and BONNIER (1882), ROMANES (1893), ITALIO GIGLIOLI (1895), HICKS and DABNEY (1897), KINZEL (1897), SANDSTEN (1898), COUPIN (1899), SCHMID (1901), DIXON (1901), SUKATSCHEFF (1901), KURZWELLY (1903), RABE (1905), BECQUEREL (1907), SCHUBERT (1909), SCHROEDER (1910), and SHULL (1913) show on the other hand that the seeds of higher plants, especially in the desiccated condition, are extraordinarily resistant to various extreme conditions and the action of various antiseptics and poisons. PRILLIEUX (1878) studied the action of carbon bisulphide gas upon the grains and found that the exposure in the gas for a week decreased the percentage of germination to 50, by 15 days' exposure to 40, and by 21 days' as low as 30. VAN TIEGHEM and BONNIER (1882) have experimented on peas, and found that the exposure to the vapour of chloroform, alcohol, and ether for two days did not destroy the vitality of the seeds. Out of thirty seeds in each lot, more than half of them germinated. ROMANES (1893) kept the seeds of mustard, red clover, beet, clover, peas, beans, spinach, cress, barley, and radish in vacuum tubes for 15 months, and many of them germinated. Some of the seeds which had been in vacuo for a period of three months, and subsequently transferred to the tubes containing oxygen, hydrogen, nitrogen, carbon monoxide, sulphuretted hydrogen, aqueous vapour, ether and chloroform gas respectively for a further period of three months, also germinated. KINZEL (1897) found in the dried seeds of peas, lupine, clover, barley, wheat, oats, and rye that two hours treatment with 0.1% formaldehyde solution was not injurious.

COUPIN (1899) showed in the desiccated seeds of wheat and clover that the exposure to the vapour of ether and chloroform for 680 hours had no influence on the latent life, but moistened seeds were killed by the same treatment. SCHMID (1901) repeated a similar experiment with the air-dried seeds of *Pisum sativum*, *Lepidium sativum*, and *Triticum sativum*, to find whether or not the chloroform vapour is permeable to the seed coat. The exposure for 24 hours already destroyed some of the seeds of pea and wheat, and after exposure for four weeks, none of the seeds germinated. The seeds of garden cress, however, were not at all injured even after a continuous exposure for two months; when the intactness of the seed coat is destroyed,

the vitality is lost within six hours. He concluded therefrom, that the chloroform vapour is just as injurious to the latent life of the seed as to the active plasm, and the resistance of the seeds to the anaesthetic gas is due to the impermeability of the seed covering to the gas and not to the latency of the plasm of the embryo as COUPIN considered. DIXON (1901) found that though the desiccated seeds of *Midicago sativa* were exposed from 10 to 30 days to the action of methyl alcohol, or to spirit saturated with mercuric chloride, or with picric acid their power of germination was not noticeably affected. Similarly the seeds of *Papaver Rhacæs*, *P. somniferum*, and *Schizopetalon Walkeri* resisted the action of spirit, but were killed by corrosive alcohol. The seeds of *Papaver Rhaeas* germinated after two days' immersion in chloroform and two days in spirit. The seeds of *Nicotiana Tabacum*, *Linaria reticulata*, *Gypsophila paniculata*, and *Calandrina umbellata*, did not germinate. He was inclined to believe that the resistance is due to the protective action of the seed coat rather than to the latency in life of the embryo, for he found that none of the punctured seed germinated by immersion in poison, whereas the intact seeds germinated.

SUKATSCHEFF (1901) observed that the sound seeds of *Lepidium mutabilis*, *Pisum sativum* in 100% and 90% alcohol for five days were not harmfully influenced. The seeds of *Lupinus luteus* were stabbed by the pen knife and allowed to stand in 90% and 100% alcohol from 1 to 18 hours, and they were found to be able to germinate. The seeds of *Lepidium sativum* similarly treated and allowed to stand from 12 to 37 hours in alcohol, germinated just as well as the control. He held the view, contrary to that held by DIXON and SCHMID, that injury to the seed coat is not a factor for losing vitality by subjecting the seed in alcohol or in chloroform.

An extensive study of KURZWELLY (1903) showed that the desiccated seeds are extremely resistant. For example, the desiccated seeds of *Sinapis alba* are able to germinate even after 541 days' immersion in alcohol, ether, benzol and carbon bisulphide. The steeping for 536 days in the above stated compounds is not fatal to the desiccated seeds of *Trifolium incarnatum*, of which 44% (carbon bisulphide) to 52% (ether) germinated. Likewise the seeds of *Trifolium hybridum*, *Ervum lens*, and *Helianthus annuus* were proved to be highly resistant. Ether and chloroform in gaseous state are

found to be more injurious than in the liquid state. The air-dried seeds of *Pisum sativum* are killed by steeping for 217 days in chloroform gas, whereas the seed in the liquid chloroform still germinated as much as 16%. After 272 days in the gas, none of the desiccated seeds germinated, whereas of those in the liquid 24% still germinated. He found that the seed covering acts as a protective in a high degree, but the reserve materials (oils and fats) diminished with the centripetal direction from the seed by the prolonged treatment in ether and chloroform, showing that the reagents finally permeated.

BECQUEREL (1907) observed on the other hand that the desiccated seed covering is impermeable to the air, various gases, alcohol, ether and chloroform, but the moistened seed coat is easily permeable and the toxic gases destroy the vitality of the seed within a short time. He came to the conclusion that the life of the embryo in the seeds of many Phanerogams can be conserved indefinitely by keeping them in a perfectly desiccated condition.

SCHUBERT (1910) extended KURZWELLY's work and showed further that poisonous substances, such as chloroform, ethyl alcohol, and amyl alcohol at their respective boiling temperatures are far more toxic than at the room temperature to the desiccated seed of *Pisum sativum*, *Sinapis alba*, *Trifolium incarnatum*, *Helianthus annuus*, *Ervum lens*, and *Setaria italica*. He conducted the experiments in such a way as to preclude the possible dissolution of the reserve materials from the seed, and arrived at results which show that the resistance of the seed against the action of reagents is due to indiffusibility of the seed coat to them, and is not concerned with the dissolution of the reserve material. The quicker the reagents penetrate, the sooner the vitality of the seed is lost.

SCHROEDER (1910) approached the subject from a practical point of view, and recommends the use of solution of silver nitrate instead of mercuric chloride for the sterilization of Grammineae seeds, for the selective-permeable membrane of the grains is impermeable to silver nitrate but easily permeable to mercuric chloride. RANSOM (1912) observed that caffeine added in the proportion from 1% to 0.01% to water in which seeds are sown, exerts a powerful effect in retarding germination and growth. If 1% is present, germination is completely inhibited. SHULL (1913) confirmed the findings of

BECQUEREL, that the dry seed covering is impermeable to dry alcohol, ether, and chloroform.

From the facts we have thus far surveyed, it is obvious that the viability of the seed is held by two main facts when it is subjected to extreme conditions, namely, the impermeability of the seed covering to certain toxic substances is one, and a state of desiccation of the seed is the other. In the forthcoming pages, we shall observe them more closely.

The selective permeability in the seed covering of rice is demonstrated as follows. The method used is the same as that used by the previous investigators. Grains of known weight are steeped in solutions of known concentrations respectively for a definite length of time, and the increase in weight of the grains due to absorption of water from the solutions of different concentration is used as a criterion of semi-permeability, for the method is shown to be quite reliable by the different authors (BROWN 1907, 1909, SHROEDER 1910, SHULL 1913). The hulls of the grains are carefully removed by the sharp scalpel, for the absorption of water by the hulls obscures the results of the ensuing experiments.

TABLE I. *Oryza sativa* ("Chiba-nishiki").

The absorption of water by the grains indicated by increase of weight in percentage. Temperature 13.5°—17° C.

Concent. No. of hours steeping.	$\frac{1}{1} N$ Na Cl	$\frac{1}{2} N$ Na Cl	Dist. water.
4.30	2.12	4.60	7.89
24	13.18	15.08	20.03
48	15.81	19.42	22.80
72	17.85	20.12	24.91
96	19.48	20.25	21.75*

\* At the end of 72 hours, the lot in distilled water is steeped in  $\frac{1}{1} N$  NaCl solution. The decrease in weight at the end of another 24 hours is shown to be due to the loss of water by exosmosis. (cf. Text fig. 1 a).

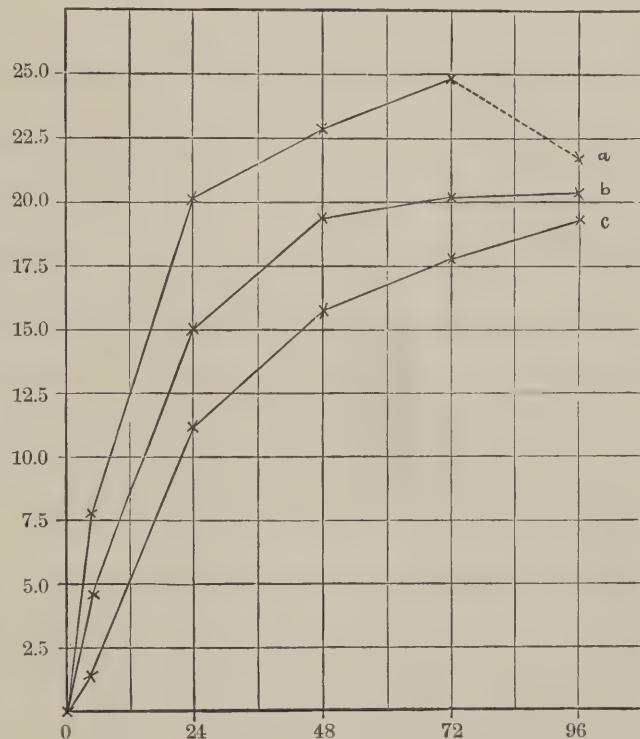
Similar data with the grains of *Zea Mays* (White Dent) show likewise the presence of a "semi-permeable" septum. The measurement at the end of 72 hours in 24—29°C is as follows:

$\frac{1}{1} N$ Na Cl	$\frac{1}{2} N$ Na Cl	Dist. water.
20.78%	26.49%	47.29%

The vitality of the grains is not concerned with the selective permeability in *Oryza* as in other grain. The hulled grains ("Kumamoto") are boiled for a few minutes with water to kill the embryo, and the semipermeability of the grain is tested. After 48 hours in the thermostat at 28°C, the increase in weight in 1 N Na Cl and in distilled water is as follows:—

1 N Na Cl	14.20%
Dist. water	30.43%

The seed covering of *Oryza* is found to be impermeable to sodium chloride, copper sulphate, mono-potassium phosphate, sulphuric acid, lithium chloride, potassium bichromate, cane sugar, oxalic acid, and ammonium oxalate from the aqueous solution, but permeable to mercuric chloride. A normal solution of mercuric chloride is absorbed just as easily as distilled water (See Table II).



Text-Fig. 1. *Oryza sativa*. Absorption of water by the grain.  
(cf. Table I). a water, b  $\frac{1}{2} N$  Na Cl, c  $\frac{1}{1} N$  NaCl. Ordinates, increase in wt.%; abscissa, no. of hours steeping.

TABLE II. *Oryza sativa.*

Increase in weight by percent.

Solution.	No. of hours steeping.	48	72
Dist. water		29.32	30.32
1 N Hg Cl <sub>2</sub>		29.20	30.73
1 N C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>		20.36	21.85
1 N COOH. COOH		19.30	21.00
1 N C <sub>2</sub> O <sub>4</sub> (NH <sub>4</sub> ) <sub>2</sub>		20.60	21.52
1 N K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>		21.41	
1 N Cu SO <sub>4</sub>		20.71	
1 N Li Cl <sub>2</sub>		21.89	

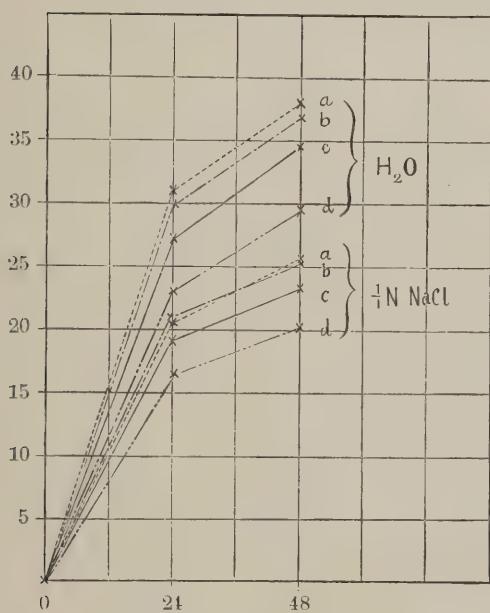
There are many factors which influence the permeability and rate of absorption of water by the living cell. The permeability of the plasm to sodium chloride is greatly changed by illumination in the leaves of lime (TRÖNDLE, 1910). The influence of light upon the permeability of the seed covering of *Zea Mays* was tested by a number of parallel tests in light and darkness at the same temperature, but no appreciable difference was found.

NILSSON-EHLE (1914) has given an evidence of the fact that the presence of a red pigment in the seed coats of wheat plays an important rôle in the water absorption and in germination. The "red" grains germinate slower than the "white," and the power of absorption of water in the former is also slower than in the latter. This physiological character seems to be correlated with Mendelian factors for red color. The seed which contains more "red factors" in its genetic constitution is slower in the rate of absorption of water than that which contains no or a less number of "red factors". The percentage of germination is also less in the seeds which contain more "red factors", and the red pigment is located in the inner layer of the integument.

In the case with rice and with some varieties of maize so far examined by the writer, the pigment is present in the pericarp instead of the integument, except a variety of maize which possesses a dark blue pigment in the aleurone layer. The absorption of water by the different varieties of maize

which contain different pigments is compared. Some of those materials were kindly sent by Prof. S. HASKELL, Massachusetts Agricultural College, Amherst, Mass. The genetic constitution of none of the materials used is known, but the result shows that the presence of red or yellow pigment in the pericarp has no retarding influence on the absorption of water. A variety of brownish yellow flint maize is found to absorb water much quicker than the rest, and a variety of dark blue flint, on the other hand, shows the slowest rate\* (cf. Text-Fig. 2).

In the case with rice, an increase in weight after 48 hours' steeping in



Text-Fig. 2. *Zea Mays*. Absorption of water by the seed. Ordinates, increase in wt.%. Abscissa, no. of hours steeping.

- a..... White dent.
- b..... Yellow flint.
- c..... Yellow dent.
- d..... Blue flint.

$\frac{1}{1}$  N NaCl and distilled water at  $16^{\circ}\text{C} - 18.5^{\circ}\text{C}$  by the red and non-pigmented grains is as follows:

"Aka-Gome"	"Chiba-Nishiki"
(pericarp red) (without red pigment)	
$\frac{1}{1}$ N NaCl 12.63%	15.25%
Dist. water 19.54%	26.07%

This shows that the red pigmented grain absorbs water more slowly than the non-pigmented. The pigment is confined to the cell wall of the pericarp, and does not belong to anthocyanin. Treating with acid or alkali, the color does not show any reaction. A prolonged treatment with absolute alcohol, ether, chloroform or hydrogen peroxide has no effect on color. Throughout the experiments the red variety ("Aka-gome") germinated

\* The grain of this variety is roundish, and the dark blue color is due to the presence of a kind of anthocyanin pigment in the aleurone cells, so that the seed becomes brilliantly red by acids and turns into blue to blue green by alkalies. This reaction can be utilized for the test of penetration of reagents through the seed covering, for if the acid diffuses into the aleurone layer, the color changes instantly to red. The grains are steeped in a  $6\text{ NH}_2\text{SO}_4$  solution for 48 hours, but many of the grains show no change in color.

always quicker than the non-pigmented ones, even if they were allowed to germinate under similar conditions. Therefore, in the case with rice, at least in the varieties examined, the rate of absorption of water and germination are not controlled by the red pigment in the pericarp.

The writer undertook a series of tests on the action of various chemical substances upon the desiccated and air dried grains of rice and maize. After the grains are steeped in the reagent for a certain length of time, they are thoroughly washed in running water at least for an hour, to remove all trace of an adhered reagent before they are placed on the germination bed. The results of the tests are tabulated and given in the Tables III-VII.

TABLE III. Germination Table.—*Oryza sativa*.

+ signs in the column indicate the condition of the grain. For example, under Hulls removed +, Desiccated +, read:—the grains are hulled and desiccated. "Ak" = "Aka-gome." "Ch" = "Chiba-nishiki." "Ar" = "Araki." "K" = "Kumamoto." "Sh" = "Shinriki."

Reagent.	No. of hours steeping.	Material.	Condition.				No. of grains.		
			Hulls removed.	With hulls.	Desiccated.	Air dry.	Germinated.	Not germinated.	Total.
Chloroform	24	"Ak."	+		+		12	4	16
	"	"Ar."		+	+		3	5	8
	"	"Ak."	+			+	0	15	15
	"	"Ch."	+			+	0	15	15
	7 days	"	+		+		0	25	25
Methyl alcohol (absolute)	21.20	"Ak."	+		+		0	15	15
	24	"	+			+	0	15	15
	21.20	"Ar."	+		+		0	15	15
	24	"Ch."	+			+	0	15	15
Formaldehyde H · CHO	21.20	"Ak."	+		+		0	15	15
	21.20	"Ar."		+	+		0	15	15
Formic acid	24	"Ak."	+		+		0	10	10
H · COOH	24	"Ak."	+			+	0	10	10

Reagent.	No. of hours steeping.	Material.	Condition.			No. of grains.		
			Hulls removed.	With hulls.	Desiccated.	Air dry.	Germinated.	Not germinated.
	"	"Ar."	+		+		0	15
	"	"Ch."	+		+		0	15
Methyl ether	24	"Ak."	+		+		0	10
$\text{CH}_3\text{-O-CH}_3$	"	"	+		+		0	10
	"	"Ar."	+		+		0	15
	"	"Ch."	+		+		0	15
Acetone	24	"Ar."	+		+		18	1
$\text{CH}_3\text{-CO-CH}_3$	"	"Sh."		+	+		20	0
	"	"Ar."	+			+	0	15
	"	"Ch."	+			+	0	15
Chloralhydrate	24	"Ak."	+		+		0	10
$\text{C Cl}_3\cdot\text{CH}(\text{OH})_2$	"	"Ar."	+		+		0	10
10% water sol.	"	"Ch."	+			+	0	20
Ethyl alcohol	24	"Ak."	+		+		14	4
(absolute)	"	"Ar."	+		+		3	15
$\text{C}_2\text{H}_5\cdot\text{OH}$	"	"Ak."	+			+	0	15
	"	"Ch."	+			+	0	15
	73	"Ak."	+		+		9	6
	"	"Ch."	+		+		13	2
7 days	"	"	+			+	0	25
Acetaldehyde	24	"Ak."	+		+		0	10
$\text{CH}_3\cdot\text{CHO}$	"	"	+			+	0	15
	"	"Ar."	+		+		0	15
	"	"Ch."	+			+	0	15
Acetic acid (glacial)	21.20	"Ak."	+		+		0	11
$\text{CH}_3\cdot\text{COOH}$	"	"Ar."		+	+		0	11
Ethyl ether	21.20	"Ak."	+		+		13	2
$\text{C}_2\text{H}_5\text{-O-C}_2\text{H}_5$	24	"	+		+		7	3
	21.20	"Ar."		+	+		0	15

Reagent.	No. of hours steeping.	Material.	Condition.			No. of grains.			
			Hulls removed.	With hulls.	Desiccated.	Air dry.	Germinated.	Not germinated.	
$\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-COOH}$	24	" Ar."	+	+	+	+	10	0	10
	"	" Ak."	+			+	13	2	15
	"	" Ch."	+			+	15	0	15
	7 days	"	+		+		16	9	25
	24	" Ak."	+		+		0	20	20
	"	" Ar."	+		+		0	20	20
	"	" Ak."	+			+	0	20	20
	"	" Ar."	+			+	0	20	20
Amyl alcohol	24	" Ak."	+		+		0	20	20
	"	" Ar."	+		+		0	20	20
Benzene	24	" Ak."	+		+		5	5	10
	"	" Ar."	+		+		7	3	10
	"	" Ak."	+			+	13	2	15
Pyridine	24	" Ak."	+		+		14	1	15
	"	" Ar."	+			+	0	10	10
	"	" Ak."	+			+	0	15	15
	"	" Ch."	+			+	0	10	10
Phenol	24	" Ak."	+		+		0	10	10
	"	"	+			+	0	10	10
	"	" Ar."	+		+		0	10	10
	"	" Ch."	+			+	0	10	10
$5\%$ water sol.	24	" Ak."	+		+		8	2	10
	"	"	+			+	2	3	5
	"	" Ar."	+		+		9	1	10
	"	" Ch."	+			+	6	8	14
$5\%$ absol. ethyl alcohol sol.	24	" Ak."	+		+		7	3	10
	"	"	+			+	9	0	9
	"	" Ar."	+		+		5	0	5
	"	" Ak."	+			+	15	0	15
$5\%$ ethyl ether sol.	24	" Ak."	+		+		7	3	10
	"	" Ar."	+		+		9	0	9
	"	" Ak."	+			+	5	0	5
	"	" Ch."	+			+	15	0	15

Reagent.	No. of hours steeping.	Material.	Condition.			No. of grains.			
			Hulls removed.	With hulls.	Desiccated.	Air dry.	Germinated.	Not germinated.	
Xylool	24	"Ak."	+		+		0	10	10
$C_6H_4(CH_3)_2$	"	"Ar."	+		+		0	10	10
"	"	"	+		+		19*	1	20
"	"	"Ak."	+		+		15*	0	15
"	"	"Ch."	+		+		14*	1	15
Picric acid.	24	"Ak."	+		+		15	0	15
$C_6H_2(NO_2)_3OII$ (saturated water sol.)	"	"	+		+		0	10	10
"	"	"Ar."	+		+		0	10	10
Thymol	24	"Ak."	+		+		14	1	15
$C_6H_3(CH_3)(C_3H_7) \cdot OH$ 10% ab. ethyl alcohol sol.	"	"Ar."	+		+		15	0	15
"	"	"Ak."	+		+		0	15	15
"	"	"Ch."	+		+		0	15	15
Resorcin	24	"Ak."	+		+		0	10	10
$C_6H_4(OH)_2$	"	"Ar."	+		+		0	10	10
5% water sol.	"	"Ak."	+		+		0	5	5
"	"	"Ch."	+		+		0	5	5
"	24	"Ak."	+		+		7	3	10
5% ab. ethyl alcohol sol.	"	"Ar."	+		+		8	2	10
"	24	"Ak."	+		+		10	0	10
5% ethyl ether sol.	"	"Ar."	+		+		8	1	9
"	"	"Ak."	+		+		5	0	5
"	"	"Ch."	+		+		16	1	17
Hydroquinone	24	"Ak."	+		+		0	10	10
$C_6H_4(OH)_2$	"	"Ar."	+		+		0	10	10
10% water sol.	"	"							
"	24	"Ak."	+		+		8	2	10
10% ab. ethyl alcohol sol.	"	"Ar."	+		+		9	1	10
Pyrogallol	24	"Ak."	+		+		2	8	10
$C_6H_3(OH)_3$	"	"	+		+		1	9	10
1% water sol.	"	"Ar."	+		+		9	1	10

\* Washed with absolute ethyl alcohol.

Reagent.	No. of hours steeping.	Material.	Condition.			No. of grains.			
			Hulls removed.	With hulls.	Desiccated.	Air dry.	Germinated.	Not germinated.	
Pyrogallop. (continued).	24	" Ch."	+			+	10	0	10
"	"	"	+		+	+	11	4	15
"	"	"	+		+	+	15	1	16
Phloroglucin. $C_6H_8(OH)_3$ conc. water sol.	24	" Ak."	+		+		10	0	10
"	"	" Ar."		+	+		10	0	10
"	24	" Ak."	+			+	11	3	14
2% water sol.	"	" Ch."	+			+	14	1	15
Anilin.	24	" Ak."	+		+		10	0	10
$C_6H_5OH$	"	" Ar."	+		+		9	1	10
"	"	" Ak."	+			+	8	2	10
"	"	" Ch."	+			+	0	10	10
Diphenylamine. $(C_6H_5)_2NH$ 10% ab. ethyl alcohol sol.	24	" Ak."	+		+		9	1	10
"	"	" Ar."	+		+		10	0	10
Acetanilide.	24	" Ak."	+		+		10	0	10
$C_6H_5NH(C_2H_3O)$ water sol.	"	" Ar."	+		+		10	0	10
"	"	" Ak."	+			+	10	0	10
"	"	" Ar."	+			+	10	0	10
"	24	" Ak."	+		+		7	3	10
"	"	" Ar."	+		+		9	1	10
1% ab. ethyl alcohol sol.	"	" Ak."	+			+	0	4	4
"	"	" Ch."	+			+	7	8	15
"	"	" Ak."	+		+		9	1	10
"	24	" Ar."	+		+		10	0	10
saturated ethyl ether sol.	"	" Ak."	+			+	10	0	10
"	"	" Ar."	+			+	10	0	10
Naphthalene.	24	" Ak."	+		+		8	1	9
$C_{10}H_8$	"	" Ar."	+		+		14	0	14
1% ab. ethyl alcohol sol.	"	" Ak."	+			+	10	10	10
"	"	" Ch."	+			+	0	15	15

Reagent.	No. of hours steeping.	Material.	Condition.			No. of grains.			
			Hulls removed.	With hulls,	Desiccated.	Air dry.	Germinated.	Not germinated.	
Naphtalene.	24	" Ak."	+		+		6	1	7
C <sub>10</sub> H <sub>8</sub>	"	" Ar."	+		+		13	0	13
5% ethyl ether sol.	"	" Ak."	+		+		1	9	10
	"	" Ch."	+		+		0	15	15
<i>a</i> -Naphthol.	24	" Ak."	+		+		10	0	10
C <sub>10</sub> H <sub>7</sub> OH	"	" Ar."	+		+		9	1	10
5% ab. ethyl alcohol sol.	"	" Ak"	+		+		0	10	10
	"	" Ch."	+		+		0	10	10
"	24	" Ak."	+		+		8	2	10
5% ethyl ether sol.	"	" Ar."	+		+		10	0	10
<i>a</i> -Naphthylamine.	24	" Ak."	+		+		9	1	10
C <sub>10</sub> H <sub>7</sub> NH <sub>2</sub>	"	" Ar."	+		+		9	1	10
10% ab. ethyl alcohol sol.	"	" Ak."	+		+		8	2	10
	"	" Ch."	+		+		3	7	10
"	24	" Ak."	+		+		9	1	10
10% ethyl ether sol.	"	" Ar."	+		+		7	3	10
Hydrogen peroxide.	24	" Ak."	+		+		14	1	15
H <sub>2</sub> O <sub>2</sub>	"	"	+		+		12	3	15
(Merk's 10%)	"	" Ch."	+		+		12	3	15
Chromic Acid.	24	" Ak."	+		+		9	1	10
1%.	"	" Ar."		+	+		6	4	10
Carnoy's Fluid.	24	" Ak."	+		+		0	15	15
Flemming's Mixture. (Ferguson's modified)	24	" Ak."	+		+		0	15	15
Flemming without acetic acid.	24	" Ak."	+		+		6	4	10
	"	" Ar."	+		+		9	1	10
	"	" Ak."*	+		+		9	0	9
	"	" *"	+		+		9	1	10

\* The grains are cut in half.

Reagent.	No. of hours steeping.	Material.	Condition.			No. of grains.		
			Hulls removed.	With hulls.	Desiccated.	Air dry.	Germinated.	Not germinated.
Flemming without acetic acid.	24	"Ch."	+			+	10	0
"	"	" *	+	+		+	9	1
Chromo-acetic mixture**	24	"Ak."	+	+	+		0	15
Hg Cl <sub>2</sub> 0.5% ab. ethyl alcohol. sol.	24	"Ch."	+	+	+		14	1
ethyl ether sol.	24	"Ch."	+	+	+		13	2
water sol.	24	"Ch."	+	+	+		0	15
Control (untreated)	24	"Ak."	+	+	+		10	0
"	"	"Ar."	+	+	+	+	10	0
"	"	"	+	+	+	+	10	0

\* The grains are cut in half.

\*\* Chromic acid 1% 1 c.c., acetic acid 1% 3 c.c., water 30 c.c.

TABLE IV. Germination Table.—*Oryza sativa*.

Reagent.	No. of hours steeping.	Material.	Condition			No. of grains.		
			Desiccated.	Air dry.	Entire hulled grain.	Cut hulled grain.	Germinated.	Not germinated.
Chloroform C H Cl <sub>3</sub>	24	"Ar."	+		+		6	4
"	"	"	+	+	+	+	0	10
"	"	"		+	+		0	10
Acetone C H <sub>3</sub> -C O-C H <sub>3</sub>	24	"Ar."	+	+	+		10	0
"	"	"	+	+	+	+	0	10
"	"	"		+	+		0	10
Absolute ethyl alcohol C <sub>2</sub> H <sub>5</sub> OH	24	"Ak."	+			+	3	10

Reagent.	No. of hours sleeping.	Material.	Condition.			No. of grains.			
			Desiccated.	Air dry.	Entire hulled grain.	Cut hulled grain.	Germinated.	Not germinated.	
Ab. ethyl alcohol (Continued)	24	"Ch."	+		+	+	8	2	10
	"	"		+	+		0	10	10
Ethyl ether $C_2H_5-O-C_2H_5$	24	"Ak."	+			+	5	5	10
	"	"Ch."	+			+	8	2	10
	"	"		+	+		8	2	10
Acetic acid $CH_3COOH$ 10% ab. ethyl alcohol sol.	24	"Ak."	+		+		6	4	10
	"	"Ch."	+		+		2	8	10
	"	"	+			+	2	4	10
	"	"		+	+		0	20	20
Resorcin $C_6H_4(OH)_2$ 5% ab. ethyl alcohol sol.	24	"Ak."	+			+	3	7	10
	"	"Ch."	+		+		8	2	10
	"	"		+	+		0	10	10
	48	"Ar."	+			+	0	10	10
	"	"	+		+		0	10	10
Hydroquinone $C_6H_4(OH)_2$ 10% in ab. ethyl alcohol sol.	24	"Ak."	+			+	4	6	10
	"	"Ch."	+		+		10	0	10
	"	"		+	+		0	10	10
Benzene $C_6H_6$	24	"Ak."	+			+	4	6	10
	"	"Ch."	+		+		9	1	10
	"	"		+	+		9	1	10
	48	"Ar."	+		+		7	0	7
	"	"	+			+	7	0	7
Naphthalene $C_{10}H_8$ 1% ab. ethyl alcohol sol.	24	"Ak."	+			+	1	9	10
	"	"Ch."	+		+		9	1	10
	"	"		+	+		0	10	10
Pyridine $C_5H_5N$	24	"Ak."	+			+	0	10	10
	"	"Ch."	+		+		0	10	10

TABLE V. Germination Table.—*Oryza sativa*.

Air dried seeds, otherwise so stated (asterisked). A=With hulls, entire grain. B=With hulls, cut, embryonal half. C=Without hulls, entire grain. D=Without hulls, cut, embryonal half.

Reagent. (Aqueous sol.)	No. of hours steeping.	Material.	Condition.	No. of grains.		
				Germ.	Not germ.	Total.
$H_2SO_4$ 6 N	21	" Ch."	A	0	25	25
	"	"	A*	26	0	26
	"	" Ar."	A	22	3	25
	"	" Ch."	B	0	25	25
	"	" Ar."	C	12	13	25
	"	" Ch."	C*	14	11	25
	"	"	D	0	25	25
$HNO_3$ 6 N	21	" Ch."	A*	0	25	25
	"	"	B*	0	25	25
	"	"	C*	0	25	25
	"	"	D*	0	25	25
$HNO_3$ 2 N	21	" Ch."	A*	0	25	25
	"	"	B*	0	25	25
	"	"	C*	0	25	25
	"	"	D*	0	25	25
$HCl$ 6 N	21	" Ch."	A*	1	24	25
	"	"	B*	0	25	25
	"	"	C*	3	22	25
	"	"	D*	0	25	25
	"	"	A	0	25	25
	"	"	B	0	25	25
	"	"	C	0	25	25
	"	"	D	0	25	25
$NaOH$ 1%	24	" Ch."	A	24	1	25
	"	"	B	1	24	25
	"	"	C	20	5	25
	"	"	D	0	25	25
$KOH$ 1%	24	" Ch."	A	25	0	25

\* Desiccated.

Reagent. (Aqueous sol.)	No. of hours steeping.	Material.	Condition.	No. of grains.		
				Germ.	Not germ.	Total.
I-KI (1% I in 4% IK)	24	" Ch."	B	6	19	25
	"	"	C	21	4	25
	"	"	D	2	23	25
(3% I in 5% IK)	24	" Ch."	A	15	10	25
	"	"	B	4	21	25
	"	"	C	0	25	25
	"	"	D	0	25	25
Cu SO <sub>4</sub> 5%	24	" K."	A	17	0	17
	"	"	B	2	21	22
	"	"	C	58	6	64
	"	"	D	7	25	32
" 10%	24	" K."	A	17	9	26
	"	"	B	9	10	19
	"	"	C	25	0	25
	"	"	D	19	6	25
KMn O <sub>4</sub> 2%	24	" Ch."	A	20	5	25
	"	"	B	21	4	25
	"	"	C	22	3	25
	"	" Ak."	C	22	3	25
	"	" Ch."	D	25	0	25
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> 10%	24	" Ch."	A	25	0	25
	"	"	B	4	21	25
	"	"	C	21	4	25
	"	"	D	5	20	25
Cr <sub>2</sub> O <sub>3</sub> 10%	24	" Ch."	A	4	21	25
	"	"	B	0	25	25
	"	"	C	0	25	25
	"	"	D	0	25	25
" 1%	24	" K."	A	27	8	35
	"	"	B	2	9	11
	"	"	C	2	15	17

Reagent. (Aqueous sol.)	No. of hours steeping.	Material.	Condition.	No. of grain.		
				Germ.	Not germ.	Total.
Os O <sub>4</sub> eti 2%	24	" K."	A	12	1	13
	"	"	B	6	1	7
	"	"	C	13	5	18
,, "	48	" K."	A	30	2	32
	"	"	B	5	1	6
	"	"	C	1	2	3
HgCl <sub>2</sub> 1%	20	" Ch."	A	0	25	25
	"	"	C	0	25	25
KCN 2%	24	" Ar."	A†	23	2	25
	"	"	B	17	8	25
	"	"	C	21	4	25
	"	"	D†	5	20	25
Pb (NO <sub>3</sub> ) <sub>2</sub> 2%	24	" Ar."	A†	25	0	25
	"	"	B†	17	8	25
	"	"	C†	23	2	25
	"	"	D†	20	5	25

† Germinated in the solution.

TABLE VI. Germination Table.—*Zea Mays*.

Y. D.=Starchy yellow dent. W. D.=Starchy white dent. Y. F.=Starchy yellow flint.

Reagent.	No. of hours steeping.	Material.	Condition.		No. of grains.		
			Desic- cated.	Air dry.	Germ.	Not germ.	Total.
Chloroform	24	Y. D.	+		0	5	5
CHCl <sub>3</sub>	"	W. D.	+		1	4	5
Methyl alcohol (absolute)	24	Y. D.	+		0	5	5
CH <sub>3</sub> OH	"	W. D.	+		0	5	5
Formaldehyde	24	Y. D.	+		0	5	5
H·CHO	"	W. D.	+		0	5	5
Formic acid	24	Y. D.	+		0	5	5
H COOH	"	W. D.		+	0	5	5

Reagent.	No. of hours steeping.	Material.	Condition.		No. of grains.		
			Desic-cated.	Air dry.	Germ.	Not germ.	Total.
Methyl ether $\text{CH}_3\text{-O-CH}_3$	24	W. D.	+		0	5	5
	"	W. D.		+	0	5	5
	24	Y. D.	+		0	5	5
	"	Y. D.		+	0	5	5
Acetone $\text{CH}_3\text{-CO-CH}_3$	24	W. D.	+		0	5	5
	"	W. D.	+		0	5	5
	24	Y. D.	+		5	0	5
	"	W. D.	+		5	0	5
Chloralhydrate $\text{CCl}_3\cdot\text{CH}(\text{OH})_2$ 10% water sol.	24	Y. D.		+	0	5	5
	"	W. D.		+	0	5	5
	"	Y. D.	+		0	5	5
	"	W. D.	+		0	5	5
Ethyl alcohol (absolute)	24	Y. D.	+		5	0	5
	"	W. D.	+		5	0	5
	"	W. D.		+	0	5	5
	"	Y. D.		+	0	5	5
Acetaldehyde $\text{CH}_3\text{-CHO}$	24	Y. D.	+		0	5	5
	"	Y. D.		+	0	5	5
	"	W. D.	+		0	5	5
	"	W. D.		+	0	5	5
Acetic acid $\text{CH}_3\text{-COOH}$ 1% water sol.	24	Y. D.	+		0	5	5
	"	W. D.	+		0	5	5
	24	W. D.	+		4	1	5
	"	Y. D.	+		4	1	5
1% ab. ethyl alcohol sol.	24	Y. D.	+		3	2	5
	"	W. D.	+		5	0	5
Butyric acid $\text{C}_2\text{H}_5\text{-O-C}_2\text{H}_5$	24	Y. D.	+		0	5	5
	"	W. D.	+		0	5	5
	"	W. D.		+	0	10	10
Amyl alcohol $\text{C}_5\text{H}_{11}\text{-OH}$	24	Y. D.	+		0	5	5
	"	W. D.	+		0	5	5

Reagent.	No. of hours steeping.	Material.	Condition.		No. of grains.		
			Desic- cated.	Air dry.	Germ.	Not germ.	Total.
Benzene	24	Y. D.	+		5	0	5
C <sub>6</sub> H <sub>6</sub>	"	W. D.	+		5	0	5
Pyridine	24	Y. D.	+		0	5	5
C <sub>5</sub> H <sub>5</sub> N	"	W. D.	+		0	5	5
	"	Y. D.		+	0	5	5
	"	W. D.		+	0	5	5
Phenol	24	Y. D.	+		0	5	5
C <sub>6</sub> H <sub>5</sub> OH	"	W. D.	+		0	5	5
5% water sol.	"	Y. D.		+	0	5	5
	"	W. D.		+	0	5	5
"	24	Y. D.	+		3	2	5
5% ab. ethyl alcohol sol.	"	W. D.	+		4	1	5
	"	Y. D.		+	0	5	5
	"	W. D.		+	0	5	5
"	24	Y. D.	+		1	4	5
5% ethyl ether sol.	"	W. D.	+		2	3	5
	"	Y. D.		+	1	4	5
	"	W. D.		+	5	0	5
Xylool	24	Y. D.	+		0	5	5
C <sub>6</sub> H <sub>4</sub> (CH <sub>3</sub> ) <sub>2</sub>	"	W. D.	+		2	3	5
Teric Acid	24	Y. D.	+		0	5	5
C <sub>6</sub> H <sub>2</sub> (NO <sub>2</sub> ) <sub>3</sub> ·OH	"	W. D.	+		0	5	5
saturated water sol.							
Thymol	24	Y. D.	+		0	5	5
(CH <sub>3</sub> )(C <sub>3</sub> H <sub>7</sub> )·OH	"	W. D.	+		0	5	5
10% in 60% alcohol sol.							
Resorcin	24	Y. D.	+		0	5	5
C <sub>6</sub> H <sub>4</sub> (OH) <sub>2</sub>	"	W. D.	+		0	5	5
5% water sol.	"	Y. D.		+	0	5	5
	"	W. D.		+	0	5	5
"	24	Y. D.	+		3	2	5
5% ab. ethyl alcohol sol.	"	W. D.	+		5	0	5
	"	Y. D.		+	0	5	5
	"	W. D.		+	0	5	5

Reagent.	No. of hours steeping.	Material.	Condition.		No. of grains.		
			Desic-cated.	Air dry.	Germ.	Not germ.	Total.
Resorcin	24	Y. D.	+		0	5	5
5% ethyl ether sol.	"	W. D.	+		4	1	5
	"	Y. D.		+	1	4	5
	"	W. D.		+	5	0	5
Hydroquinone $C_6H_4(OH)_2$	24	Y. D.	+		0	5	5
10% water sol.	"	W. D.	+		0	5	5
"	24	Y. D.	+		4	1	5
10% ab. ethyl alcohol sol.	"	W. D.	+		4	1	5
Pyrogallol	24	Y. D.	+		0	5	5
$C_6H_3(OH)_3$	"	W. D.	+		1	4	5
2% water sol.	"	Y. D.		+	0	5	5
	"	W. D.		+	3	2	5
Phloroglucin	24	Y. D.	+		0	5	5
$C_6H_3(OH)_3$	"	Y. D.		+	0	5	5
2% water sol.	"	W. D.	+		3	2	5
	"	W. D.		+	2	3	5
Anilin	24	Y. D.	+		2	3	5
$C_6H_5NH_2$	"	Y. D.		+	1	4	5
	"	W. D.	+		2	3	5
	"	W. D.		+	1	4	5
Diphenylamine $(C_6H_5)_2NH$	24	Y. D.	+		3	1	5
10% absolute alcohol sol.	"	W. D.	+		4	1	5
Acetanilide	24	Y. D.	+		5	0	5
$C_6H_5NH(C_2H_3O)$	"	W. D.	+		4	1	5
1% water sol.	"	Y. D.		+	4	1	5
	"	W. D.		+	5	0	5
"	24	Y. D.	+		3	2	5
saturated ethyl ether sol.	"	W. D.	+		5	0	5
	"	Y. D.		+	4	1	5
	"	W. D.		+	5	0	5
Naphthalene $C_{10}H_8$	24	Y. D.	+		3	2	5
5% ethyl ether sol.	"	W. D.	+		5	0	5

Reagent.	No. of hours steeping.	Material.	Condition.		No. of grains.		
			Desic- cated.	Air dry.	Germ.	Not germ.	Total.
Naphthalene	24	Y. D.		+	0	5	5
(Continued)	"	W. D.		+	5	0	5
"	24	Y. D.	+		4	1	5
1% ab. ethyl alcohol sol.	"	W. D.	+		5	0	5
"	"	Y. D.		+	0	5	5
"	"	W. D.		+	0	5	5
$\alpha$ -Naphthol	24	Y. D.	+		2	3	5
$C_{10}H_7OH$	"	W. D.	+		3	2	5
5% ab. ethyl alcohol sol.	"	Y. D.		+	0	5	5
"	"	W. D.		+	0	5	5
"	24	Y. D.	+		0	5	5
5% ethyl ether sol.	"	W. D.	+		2	3	5
$\alpha$ -Naphtylamine	24	Y. D.	+		4	1	5
$C_{10}H_7NH_2$	"	W. D.	+		4	1	5
10% ab. ethyl alcohol sol.	"	Y. D.		+	0	5	5
"	"	W. D.		+	0	5	5
"	24	Y. D.	+		0	5	5
10% ethyl ether sol.	"	W. D.	+		2	3	5
Hydrogen peroxide	24	Y. D.	+		5	0	5
$H_2O_2$	"	W. D.	+		4	1	5
$HgCl_2$	24	Y. D.	+		4	1	5
0.5% ab. ethyl alcohol sol.	"	W. D.	+		5	0	5
"	24	Y. D.	+		2	3	5
0.5% ethyl ether sol.	"	W. D.	+		4	0	4
"	24	Y. D.	+		0	5	5
0.5% water sol.	"	W. D.	+		0	5	5

TABLE VII. Germination Table.—*Zea Mays*.

Air-dried grain, otherwise so stated (asterisked).

Reagent. (Aqueous sol.)	No. of hours steeping.	Material.	Germinated.	Not germ.	Total.
$H_2SO_4$ 6 N	24	Y. D.	0	5	5
	"	W. D.*	5	0	5
	"	Y. F.	0	5	5
$HNO_3$ 6 N	21	Y. D.	0	5	5
	"	W. D.*	0	5	5
	"	W. D.	0	5	5
	"	Y. F.	0	5	5
$HNO_3$ 2 N	21	Y. D.	0	5	5
	"	W. D.*	0	5	5
	"	W. D.	0	5	5
	"	Y. F.	0	5	5
HCl 6 N	21	Y. D.	0	5	5
	"	W. D.*	4	1	5
	"	W. D.	0	5	5
	"	Y. F.	0	5	5
NaOH 1%	24	Y. D.	0	5	5
	"	W. D.	0	5	5
	"	Y. F.	0	5	5
KOH 1%	24	Y. D.	0	5	5
	"	W. D.	0	5	5
	"	Y. F.	0	5	5
I-KI. (1% in 4% IK)	24	Y. D.	0	5	5
	"	W. D.	0	5	5
	"	Y. F.	0	5	5
$CuSO_4$ 10%	24	Y. D.	1	4	5
	"	W. D.	0	5	5
	"	Y. F.	5	0	5
KMnO <sub>4</sub> 1%	24	Y. D.	5	0	5
	"	W. D.	4	1	5
	"	Y. F.	4	1	5

\* Desiccated.

Reagent. (Aqueous sol.)	No. of hours steeping.	Material.	Germinated.	Not germ.	Total.
$K_2 Cr_2 O_7$ 10%	24	Y. D.	0	5	5
		W. D.	0	5	5
		Y. F.	1	4	5
$Cr_2 O_3$ 10%	24	Y. D.	0	5	5
		W. D.	0	5	5
		Y. F.	0	5	5

The more important points brought out in the preceding tables are as follows:

(1) The desiccated grains of *Oryza* and *Zea* are far more resistant than air dried ones. To the air dried *Oryza* grain twenty four hours' steeping in chloroform, acetone, commercial absolute ethyl alcohol, picric acid (saturated solution), absolute ethyl alcoholic solution of thymol, naphthalene,  $\alpha$ -naphthol, and twenty one hours' steeping in 6N sulphuric acid are fatal, whereas the same treatment on the desiccated grain is only slightly noxious or entirely harmless. Likewise in the case with *Zea*, commercial absolute ethyl alcohol, the absolute ethyl alcohol solution of naphthalene (1%), resorcin (5%),  $\alpha$ -naphthol (5%),  $\alpha$ -naphthylamine (10%), 6N sulphuric acid and hydrochloric acid are fatal to the air dried grain, but not to the desiccated one.

(2) The following treatment is proved to be fatal both to desiccated and air-dried grains of *Oryza* and *Zea*: steeping them for twenty four hours in commercial absolute methyl alcohol, formaldehyde, formic acid, methyl ether, chloralhydrate (10% aqueous solution), acetaldehyde, acetic acid, butyric acid, amyl alcohol, pyridine, phenol (5% aqueous solution), resorcin (5% aqueous solution), hydroquinone (10% aqueous solution), and nitric acid (3N, 6N).

(3) Twenty four hours' steeping in an aqueous solution of phenol (5%), resorcin (5%), acetic acid (1%), and sublimate (0.5%) is fatal to both desiccated and air-dried *Oryza* grains, whereas the corresponding absolute alcoholic or ether solutions are much less injurious. The case with acetic acid, perhaps, is of interest. The fixing reagents for plant tissues commonly used in the histological and cytological practices, such as chromo-acetic mixture, Carnoy's and Flemming's fluids, contain a certain amount of acetic acid. An

aqueous solution of each component of these mixtures, i.e. osmic acid and chromic acid, except acetic acid, is not fatal. Twenty four hours' steeping, which is the usual length of time for fixing ordinary tender tissues, in Fleming's mixture without acetic acid (BENDA'S mixture, for example, recommended for the chondriosome studies, contains no acetic acid) hardly kills the embryo of the air-dried rice grains. An aqueous solution of acetic acid alone, however, kills the desiccated grains, but an absolute alcoholic and ether solution of the same strength are not fatal; many of the grains, even when they are cut in half and steeped for 24 hours, have proved capable of germination.

(4) The resistance of the desiccated *Oryza* grain against toxic solutions within a limited length of time is also pronounced even with the grain cut in half near the embryo at the endosperm, as in the entire grain. As we see in Table IV, many of the desiccated cut grains retained their viability after twenty four hours' steeping in ethyl alcohol, ethyl ether, absolute alcoholic solution of resorcin, acetic acid, hydroquinone, and naphthalene. In the last case, however, only one grain out of ten germinated, whereas none of the air-dried whole grain, except in the case of ethyl ether, germinated.

Regarding the cause of viability opinions among previous authors are somewhat diverse. Some consider that the presence of the impermeable membrane in the seed coats is the chief factor (SCHMID 1901, DIXON 1901), but SUKATSCHEFF (1901) doubts this. SUKATSCHEFF finds that the vitality of the seed is not destroyed by the action of antiseptics, even when the seed-covering is broken; so he thinks, that if the presence of the seed coat is the only factor for the resistance of the dormant seed against the antiseptics, the explanation is questionable. The findings in the *Oryza* grains also show that the desiccated cut grains are in many cases just as resistant as the entire ones. Whole air-dried grains, however, are killed by the same treatment, showing that the presence of perfect coating is not the only cause of resistance, but that some other factors are at work. We may consider at least three possible causes of increased resistance of the desiccated seed, namely (1) an increase in the protective action of the selective permeable septum, (2) an increase in the filtering power of the endosperm tissue, and (3) an increase in the stability of the plasm of the embryo.

These factors may act independently or combined according to the case, so that the selective septum plays only a part, which, however, can by no means be overestimated.

## II. The Seat of the Selective Permeable Septum in the Seed Covering.

In spite of the fact that the selective permeability displayed by the seed is well marked, the localization of the septum in the seed covering is not quite definitely known. BROWN (1907, 1909) considers, that in the case with *Hordeum vulgare* var. *caeruleascens*, the selective power is confined to the testa, and probably to that portion which is derived from the epidermis of the nucellus during the development of the seed. He studied the limit of penetration by treating the grain with a solution of silver nitrate for 48 hours, then in a solution of sodium chloride. The precipitation of silver chloride in the tissue and the subsequent blackening on exposure to light makes the limit of penetration visible. By this method, he found that a part of the spermoderm is colored, but a thin layer of uncolored membrane remains between the stained portion and the walls of the aleurone cells. SCHROEDER (1911) thinks, however, that in the wheat grain, it is the cutinized or lignified inner integument, instead of the epidermis of the nucellus, which gives the typical cellulose reaction and dissolves promptly in concentrated sulphuric acid. The present study in *Oryza* agrees with SCHROEDER's observation on *Triticum*, namely, that the selective permeable septum in the seed of *Oryza* is the thickly cutinized inner wall of the inner integument, and probably the case is also true in *Zea*.

It is highly desirable to obtain, at the outset, a definite knowledge in the anatomy of the seed coat, but unfortunately the descriptions given by previous authors disagree. According to VOGL (1899) the pericarp ("Silberhäutchen") of the rice grain consists of (1) "Epidermis", (2) "Schwammparenchym", "Querzellenschicht", and (3) "Schläuche", but the true seed coat is not observable („Samenhaut als besondere Zellschicht ist noch nicht nachweisbar“). The figure that he gives (p. 130) appears to be quite different from what we actually observe in our material. OWAKI (1902) gives a some-

what different account. The pericarp consists of (1) epidermis, (2) parenchyma of five to six cell layers, (3) chlorophyll layer of two cell layers, and (4) tube cells. The spermoderm (seed coat) consists of (5) three to four layers of cells when the seed is yet young, but in the matured grain, only one cell layer is observed.

Likewise in the case with wheat, statements by the different authors do not agree. NILSSON-EHLE (1914) is led to consider that the statement which we find in the standard text-books seems to require some revision. According to them, the seed coat of wheat consists only of two cell layers, derived from the inner integument, but what he actually finds, is that the seed coat consist of two entirely independent layers which are insoluble in concentrated sulphuric acid, and in the case with the 'red' seed, these two layers are present, but in the case with 'white' seed, the inner layer is resorbed.

The writer made no attempt to trace the development of the ovary in rice; the investigation has only been confined to the young and fully matured grains of the agricultural varieties known as "Hassaku", and "Kumamoto", both being the upland rice and grown in the University Farm. The material is fixed with a chromo-acetic mixture, or Flemming's weak solution and imbedded in paraffine. The microtome sections are stained with iron-alum haematoxylene or the triple staining with safranine, gentian violet and orange G. The following layers are clearly observed in the matured grain: The seed covering consisting of (1) epidermis with a thin cuticula layer, (2) parenchym comprised of ten to twelve cell layers, (3) chlorophyll layers (two cell layers), (4) tube cells, which lie transversely to the previous cell layers (Compare Pl. IX, figs. 1-2). It is almost impossible to observe definitely the true structure of the seed coat in the grain, even in the milk-ripe stage. The anatomical positions to which the remaining existing tissues in the matured seed covering should properly be placed, can only be defined by following up the development of the ovary from a very early stage. In the milk-ripened grain only two distinct cell layers are observed between the tube cells and the aleurone layer, and these two layers must be a part of the integument and the nucellus. A thick cutinized layer is found between these two layers (Pl. IX fig. 1-2, c), and in a fully matured grain the cutinized layer

appears to lie directly above the aleurone layer, for the lower layer (Pl. IX fig. 1-2, *n*) is resorbed. It is a difficult task to ascertain, whether the cutinized layer is derived from the upper cell layer or from the underlying cell layer. For this point, the writer is indebted to Dr. KUWADA, who kindly allowed the writer to refer to the slides and the drawings made by him in his cytological studies on *Oryza*. At a very early stage in the development of the embryo, both the inner and outer integuments are clearly seen and the inner wall of the inner integument, which faces the nucellar epidermis, is cutinized. The cutinized layer observed in the matured grain, then, must be considered to have been derived from the upper cell layer which must be the inner layer of the inner integument; accordingly, the underlying cell layer must belong to the nucellus.

The green colour of the immature grain is due to the presence of many chlorophyll bodies in the parenchym of the pericarp, and they are rich in starch grains. As maturity advances, the chlorophyll bodies disappear, except in the two slender "chlorophyll cell" layers, and finally the chlorophyll bodies are lost even from the latter. The tube cells, which are loosely attached, are arranged along the longitudinal axis of the grain, so that the transverse section of the grain gives their cross sections (Pl. IX, fig. 1, *t*). The tissue above the tube cells (the pericarp) can be peeled off from the remaining tissue (the spermoderm), and the majority of the tube cells remain on the part of the spermoderm.

The following microchemical tests were made with fresh and fixed materials. The parenchym of the pericarp gives a reaction of cellulose by potassium teriodide (I-KI) with concentrated sulphuric acid. Cupra ammonia dissolves them slowly. No lignin reaction is observed either by phloroglucin or by  $\alpha$ -naphthylamine with a strong solution of hydrochloric acid, except in the wall of tracheid of the vascular bundle. According to NOWACKI (1870), however, the chlorophyll-containing single cell layers of the pericarp and the underlying tube cells in wheat grain are lignified. This is not the case with rice grain. The cell wall of the chlorophyll layers and the tube cells dissolve in a concentrated solution of sulphuric acid, and by an addition of I-KI, a deep blue color is produced.

A solution of ruthenium-red stains the cell wall of epidermis,

parenchyma of the pericarp and the aleurone layer, suggesting the presence of a pectine substance. A thin cuticula layer is found at the upper epidermis of the pericarp and a thick layer at the inner wall of the inner integument.

The cell wall of the spermoderm is dissolved by the addition of concentrated sulphuric acid, except the cutinized layer.

As already referred to, the cuticula layer in the matured grains appears to lie directly above the aleurone layer, but in maceration by a strong solution of sulphuric acid with iodine, the cuticula and its component cell wall is separated, the former being colored brown, and the latter dissolving in a deep blue colour. A weak solution of sodium hydroxide easily destroys the seed-covering, especially along the vascular bundles which lie along the dorsal ridge of the grain.

The fact that the cutinized layer of the inner integument withstands the action of strong acids, leads to an assumption that this layer may be the seat of the selective permeable septum. SCHROEDER (1911) has already expressed this view. The *Oryza* grains are steeped in a strong sulphuric acid or hydrochloric acid solution for 24 hours and then thoroughly washed. A thin section of the treated grains is mounted on a slide with a drop of Congo red or orange G. The section shows, that the acid penetrates the tissue which lies above the cuticula layer, but in the tissue which lies below the cuticula, no acid reaction is observed.

Furthermore, the results of the experiments with the dye solutions support the above assumption. A majority of the dye stuffs studied, dissolving in various solvents with various concentrations, stain only the tissue above the cuticula layer of the integument, but the underlying tissue remains unstained. In most cases, a sharp demarkation is made at the cutinized layer. The dyes used for the experiments are the following. Those which are marked with signs are known as vital staining ones.

Gentian violet\*§

Dahlia\*

Malachite green F. S.\*‡

Methyl green\*§‡

Aniline blue\*

Light green S. F.†

Thionine\*

Bismarck brown\*§

\* by OVERTON (1900), § by PFEFFER (1886), † by RUHLAND (1912), ‡ by HÖBER (1909).

Congo red	Orange G.†
Methyl orange§	Magdala red
Safranine*§	Induline
Eosin	Rose bengale
Fluorescein	Galein sicc.
Coccinin	Bordeaux R
Tropaeolin OO	Tropaeolin OOO
Haematein	Nigrosine
Cyanine§	Orcëin
Fuchsine§	Methylene blue‡
Sudan III	Alkanine

After the hulled grains are steeped in the dye solution for a certain length of time, they are pressed between clean blotting paper to remove the adhered solution, and the limit of penetration is examined under the microscope, by hand sections of single grains from each lot (usually five in each lot).

The results are as follows:

\* by OVERTON (1900), § by PFEFFER (1886), † by RUHLAND (1912), ‡ by HÖBER (1909).

TABLE VIII.

Permeability of the cuticular layer of the seed — covering to the dyes. + : permeable;  
— : impermeable.

Dye stuffs	Cone.%	Solvent	No. of days steep.	Oryza	Zea	Hordeum
Alkanine	2.0	Chloroform + alcohol (95%)	1	—		
"	"	"	5	—		
Aniline blue	0.25	$\frac{1}{40}$ N Na OH	2	—		
"	"	$\frac{1}{100}$ N "	"	—		
"	"	$\frac{1}{1000}$ N "	"	—		
Bismarek brown	0.1	dist. water	1	—		
Bordeaux R	0.1	dist. water	1	—		
Coccinin	0.05	dist. water	1	—	—	
"	0.05	"	"	—	—	
"	"	50% methyl alcohol	2	—	—	—
"	0.1	dist. water	1	—	—	
"	"	"	2	—	—	—
"	"	50% ethyl alcohol	"	—	—	—
"	"	1% Phloroglucin	1	—	—	
"	"	1% Hg Cl <sub>2</sub>	"	—	—	
"	0.25	20% Na <sub>2</sub> CO <sub>3</sub>	2	—		
"	"	$\frac{1}{40}$ N Na OH	"	—		
"	"	$\frac{1}{100}$ N "	"	—		
"	"	$\frac{1}{1000}$ N "	"	—		
"	0.1	Chloroform	7	—		
Congo red	0.005	dist. water	1	—	—	
"	0.05	"	"	—	—	
"	0.1	"	"	—	—	
"	"	"	2	—	—	—
"	"	50% ethyl alcohol	"	—	—	—
"	0.05	50% methyl alcohol	"	—	—	—
"	0.1	Phloroglucin	1	—		
"	"	1% HgCl <sub>2</sub>	"	—		
Cyanine	0.1	95% ethyl alcohol	2	—		
"	0.05	45% ethyl alcohol	3	—		

Dye stuffs	Cone.%	Solvent	No. of days steep.	<i>Oryza</i>	<i>Zea</i>	<i>Hordeum</i>
Dahlia	0.1	dist. water	1	—	—	
"	"	90% alcohol	7	—		
Eosin	0.1	dist. water	2	—	—	—
"	"	50% ethyl alcohol	"	(+)	+	(+)
Fluorescein	0.05	47.5% ethyl alcohol	1	+	+	
"	0.1	50% ethyl alcohol	"	+		
"	"	"	2	+	+	
"	0.1	47.5% ethyl alcohol	"			+
"	0.1	90% ethyl alcohol	1	+		
"	"	Ammoniacal water	"	—	—	
"	"	"	4	+		
Fuchsine	0.1	dist. water	2	—		
Galein sicc.	0.1	50% ethyl alcohol	1	+	+	
"	"	90 " "	2	+		
"	"	Ammoniacal water	1	—	—	
"	"	47.5% ethyl alcohol	2			+
Gentian violet	0.005	dist. water	1	—		
"	0.05	"	"	—		
"	"	"	2	—	—	
"	"	50% methyl alcohol	"	(+)?	+	—
"	0.1	45% ethyl alcohol	1	—	—	
Haematein	0.1	90% ethyl alcohol	2	—		
Induline	0.1	90% alcohol	7	—		
"	"	dist. water	2	—	—	
Light green F. S.	0.005	dist. water	1	—	—	
"	0.05	"	"	—	—	
"	"	50% methyl alcohol	2	—	—	
"	"	50% ethyl alcohol	"	—	—	
"	"	dist. water	"	—	—	
"	0.1	"	1	—	—	
"	0.25	1/40 N Na OH	2	—		
"	"	1/100 "	"	—		
"	"	1/1000 "	"	—		
"	"	20% Na-Carbonate	"	—		

Dye stuffs	Cone, %	Solvent	No. of days steep.	Oryza	Zea	Hordeum
Light green F. S.	0.1	90% ethyl alcohol	3	—		
Magdala red	0.1	dist. water	2	—		
"	"	"	1	(+)?	—	
Malachite green	0.1	dist. water	1	—	—	
"	"	"	2	—	—	
"	"	50% ethyl alcohol	"	—	+	
"	"	90% ethyl alcohol	3	—		
Methyl green	0.1	dist. water	3	—		
"	conc.	Acetic acid	1	—	—	
"	0.1	1% " "	2	—		
"	"	50% ethyl alcohol	"	—	+	—
"	"	dist. water	"	—	—	
Methylene blue	0.1	dist. water	2	—	—	—
"	"	50% ethyl alcohol	"	—	+	
"	0.05	50% methyl alcohol	"	—	+	—
"	"	dist. water	"	—	—	
Methyl orange	0.1	dist. water	2	—		
Nigrosine	0.1	dist. water	2	—		
"	"	"	4	(+)?		
Orange G.	0.005	dist. water	1	—	—	
"	0.05	"	"	—		
"	0.25	20% Na-bicarbonate	2	—		
Orcéin	0.1	95% ethyl alcohol	2	—		
"	0.05	45% " "	1	—		
Rose bengale	0.1	dist. water	3	—		
"	"	50% ethyl alcohol	2	—	—	
"	"	dist. water	"	—		
"	0.05	50% methyl alcohol	"	(+)?	—	—
Safranine	0.05	dist. water	1	—	—	
"	"	50% ethyl alcohol	"	+		
"	conc.	aniline water	"	+	+	
Sudan III	2	95% ethyl alcohol	5	—		

Dye stuffs	Conc.%	Solvent	No. of days steep.	<i>Oryza</i>	<i>Zea</i>	<i>Hordeum</i>
Thionine	0.1	dist. water	2	—	—	—
"	"	50% ethyl alcohol	"	—	+	+
"	0.05	50% methyl alcohol	"	(+)?	+	
"	"	dist. water	"	—	—	
"	0.1	50% methyl alcohol	"			+
Thionine + Coccinin	0.1	dist. water	3	—		
Thionine + Light green	0.1	dist. water	3	—		
Tropaeolin OO	0.05	47.5% ethyl alcohol	3	—		
"	0.1	95% "	2	—		
Tropaeolin OOO	0.1	dist. water	1	—		

One of the interesting facts observed, however unimportant it may be in this connection, is the influence of the solvent on the permeability of the seed-covering. Fluorescein is an insoluble dye stuff in cold water, but easily soluble in hot water or in a cold weak alkaline solution, as well as in a strong alcohol solution. A weak ammoniacal solution (0.1%) of this dye does not pass through the septum, but does if applied in an aleoholic solution (dissolved at first in a small amount of absolute alcohol and made up to a desired concentration by an addition of water). An aqueous solution of safranine (0.05%) is indiffusible, but its ethyl-alcoholic solution is diffusible. A concentrated aniline-water solution is also diffusible. The septum in *Zea* is permeable to methylalcoholic solution (0.05%) of gentian violet, Malachite green, methylene blue, methyl green, and thionine, but impermeable to the respective aqueous solutions. While that of *Oryza* is impermeable to either aqueous or to alcoholic solutions of gentian violet, Malachite green, methyl green, and methylene blue.

The permeability of the seed-covering by the solvent is independent of the permeability by the dissolved dyes. For example, it is easily permeable to acetic acid but impermeable to methyl green dissolved in a weak acetic acid solution, just as in the case with an aqueous solution. A distinct demarkation is made by the cuticular layer; above it is intensely stained and the underlying tissue is utterly without staining. Coccinin, Congo red,

cyanine, dahlia, light green F. S., and methyl green dissolved in a weak alcohol do not enter, while alcohol easily does and destroys the vitality of the seed.

From these facts, we may safely assume that the cutinized layer which is derived from the inner integument is the seat of the septum which excludes the passage of the chemical compounds selectively.

### III. Rôle of Oxygen in Germination.

It is shown by CROCKER (1914) on *Alisma* and by TAKAHASHI (1905) on *Oryza* that the seed of these plants can be germinated in the absence of air. The latter author placed the sterilized unhulled grains in a vessel which was filled with thoroughly boiled water and sealed up by means of a layer of mercury. After five days germination was observed, and during 36 days out of 2.3966 gr. of initial weight of grains, 0.4354 gr. of starch was lost by intermolecular respiration. He found bacterial infection in spite of sterilization performed by a one-per-mille solution of corrosive sublimate for one hour and half. It is uncertain if this short treatment is effective on the unhulled grain, for it has been observed that the steeping of the unhulled grains in an aqueous solution of potassium permanganate for more than 24 hours has no effect inside the hulls, whereas hulled grains are totally destroyed by the same solution within six hours. Furthermore, a trace of air might be inclosed inside the hulls, so it seems better to use the hulled grains for the experiment.

By several experiments, using hydrogen gas and a solution of potassium pyrogallate, the writer verified the statements of previous investigators, that the rice grain needs only extremely low oxygen pressure for the germination. The soaked grains are able to bring the embryos to germinate by intermolecular respiration in an atmosphere of hydrogen gas, or in air from which the oxygen has been removed by potassium pyrogallate. In addition, it was observed that under the anaerobic condition, the development of the plumule is possible, but that of the radicle is entirely suppressed. It is peculiar to *Oryza* that the plumule appears first in germination, contrary to the majority of Gramineae, in which the development of the radicle precedes that of the

plumule. YOKOI (1898) and AKEMINE (1913) show, however, that the development of the radicle precedes, if the grains are allowed to germinate under a scarcity of available moisture. According to YOKOI, the plumule alone develops two or three days before the radicle develops, if the grains are allowed to germinate in a sufficient supply of water; but when the quantity of water contained in the medium (sand bed) is scanty (15%—7.5%), the radicle develops before the plumule appears.

Fifty air-dried hulled grains ("Shin-Riki") are divided into four lots. Two lots are allowed to germinate in free access of air, and the other two lots are allowed to germinate under anaerobic conditions. One of the two lots in each is steeped in water (10 mm. deep), and the other is placed on the moist filter paper. The grains are put in a vial which is placed in a glass cylinder of a capacity of ca. 300 c.c. with a tight fitting glass stopper. 30 c.c. of oxygen absorbent (1 gr. pyrogallol + 5 gr. KOH + 30 c.c. H<sub>2</sub>O), freshly prepared, are poured into the cylinder, which is immediately closed with the stopper and subsequently sealed up with melting paraffine. As control, 30 c.c. of distilled water instead of the oxygen absorbent is given and sealed up likewise. They are kept in the thermostat at 24°C. After 20 hours, germination had not yet taken place; at the end of 41 hours, the grains both in water and on the filter paper, in the ordinary air, germinated. In the oxygen free air, however, the grains on the filter paper germinated, but those in water still remained ungerminated. No growth of root hairs is found on the rootlets grown in water, but on those grown on the filter paper, they are abundantly formed (cf. KLEBS 1884 p. 570-571). At the end of five days, the chambers are opened, and the measurement of the length of the shoots and roots is made. The result is as follows (Table IX):—

TABLE IX. *Oryza sativa* ("Shin Riki").

The influence of oxygen on the development of root and shoot in seedlings.

	Germinated in ordinary air.		Germinated in oxygen free air.	
	Root m.m.	Shoot m.m.	Root m.m.	Shoot m.m.
Germinated on the moist filter paper	22	10	less than 1	16
	14	5	"	16
	15	14	"	14
	16	8	"	21
	12	6	"	17
	22	17	"	20
	12	7	"	16
	20	12	"	16
	17	13	"	15
	15	8	"	17
	21	15	"	19
	15	8	"	20
	27	15	"	12
	15	7	"	24
	13	11	"	14
	av. 17.06		10.4	
	5	37	less than 1	22
Germinated in 10 mm. deep distilled water	7	43	"	24
	4	37	"	22
	3	35	"	21
	8	46	"	30
	6	36	"	24
	3	21	"	24
	3	34	"	24
	4	39	"	25
	5	37	"	20
	1	25	"	22
	9	41	"	22
	2	33	"	24
	7	44	"	22
	6	37	"	34
av. 4.86		36.33		less than 1
		23.33		

By transferring the seedlings from the water to the moist-air chamber, the rate of development of the root becomes vigorous, while that of the shoot is very gradual. Twenty hulled grains ("Araki") are allowed to germinate in distilled water at 27°C. At the end of 48 hours the average length of shoots was 10.55 mm; one of the grains was found to have a rootlet of 2 mm in length, the rest had none. After the measurement was made, the seedlings were placed on a moist filter paper and allowed to grow another 24 hours at 27°C. Every one of them developed the root, and the average length reached was 11.65 mm. (Table X).

TABLE X. *Oryza sativa*.

Influence of oxygen on the development of radicle.

	Growth in length mm.	
	first 48 hrs. in water	next 24 hrs. in air
Root	0.1	11.55
Shoot	10.55	2.60

The result is more pronounced in the case where the grains are brought to normal air from the oxygen free air in which they have been allowed to germinate (Table XI).

TABLE XI. *Oryza sativa*, same as Table X.

	Growth in length mm.		
	First 48 hrs. in oxygen free air	Next 48 hrs. in	
		oxy. free air	normal air
Root {	in water	0	0
	on moist filter paper	0	10.3
Shoot {	in water	less than 1	12.8
	on moist filter paper	,,	14.3

As we see from the above figures, the development of the radicle vigorously commences as soon as oxygen becomes available. No growth of

radicles observed in the seedlings, which remained in the oxygen free air at the end of 96 hours, while the radicles reached a length of as much as 10.3 mm. (average of twenty grains) in the seedlings which were removed from the oxygen-free air at the end of 48 hours, exposed to the ordinary air and allowed to stay another 48 hours.

The results are verified by another set of experiments, using hydrogen gas for the replacement of air and with lower atmospheric pressure through partial removal of the air by means of a suction pump. The development of the radicle is dependent on the amount of available oxygen present. If the air is partially replaced or removed, the radicle develops only very little, and with a complete absence of available oxygen no development of the radicle becomes possible.

The data above given show that the development of the radicle is dependent on the oxygen supply, whether the grains are submerged in water or placed on the moistened filter paper to supply a sufficient amount of moisture necessary for germination. As soon as the germinated grains, in which the development of the radicle is checked by lack of oxygen, are allowed to be accessible to the ordinary air, the radicle begins to develop.

Many years ago, KLEES (1884, p. 599) gave a biological interpretation of the absence of root hairs and the vigorous elongation of shootlets in *Oryza* seedlings. He considered these facts as examples of the adaptation of water plants to the environment. We have seen that the development of root hairs and the rapid elongation of the rootlets take place if the grains are germinated in moist air, instead of in water. The phenomena are simply due to the influences of external conditions. Deficiency in the oxygen supply in submerged condition is the vital cause of the slowness in the growth of the radicle, thus on the removal of this limiting factor the growth commences rapidly, as has already been observed.

#### **IV. The Effect of H and OH Ions on Germination.**

The stimulating action of H and OH ions on the germination of the seed in *Sagittaria* is reported by A. FISCHER (1907), but his result is not verified by CROCKER (1907, '14). In the germination of the grains of *Oryza*,

there appears to be no appreciable stimulating influence of OH or H ions. The hulled grains ("Kumamoto") are placed in  $\frac{1}{10}$  N  $\text{H}_2\text{SO}_4$ ,  $\frac{1}{10}$  N,  $\frac{1}{20}$  N oxalic acid,  $\frac{1}{10}$  N,  $\frac{1}{20}$  N NaOH, and distilled water. Each culture received 100 c.c. of the solution and fifty grains. At the end of 24 hours in the thermostat at  $27^\circ\text{C}$  no germination is observed, except the controls (distilled water) in which the plumule slightly appeared. The concentration of NaOH, as low as  $\frac{1}{10}$  N, collapses the seed covering, the grains thus being destroyed. No injury is observed in other cultures. At the end of 72 hours, still no germination took place, except in distilled water. The germinated grains developed plumules over 1.5 cm in length. Even after five days, no germination was observed in the solutions, showing that the grains of *Oryza* are unable to germinate in acidity or alkalinity as low as  $\frac{1}{20}$  N. FISCHER, however, found stimulating action in *Sagittaria* in the  $\frac{1}{10}$  N solutions. Consequently lower concentration, were applied, namely:  $\frac{1}{50}$ ,  $\frac{1}{100}$ ,  $\frac{1}{1000}$  N oxalic acid and NaOH, together with tap and distilled water as controls. At the end of 24 hours no germination had begun, but the embryos swelled slightly, except with distilled water, in which the swelling was distinct. At the end of 48 hours germination took place in all cultures; no marked difference could be observed, which may be attributed to the action of the applied ions. After four days, the average length of the shootlets in the seedlings were measured as follows:

Na OH $\frac{1}{50}$ N	less than 1.0 mm.
„ $\frac{1}{100}$ N	1.4 mm.
„ $\frac{1}{1000}$ N	10.0 mm.
Tap water	9.9 mm.
Distilled water	15.0 mm.

Again, the development is found to be best in distilled water. A question may arise whether the distilled water might have contained a trace of copper or some other heavy metal ions which act as stimulants for the germination. The control experiments,\* however, show negative results, so

\* The following cultures were included:

1. Redistilled water (hard glass wares used) in paraffined hard glass Erlenmeyer flasks.
2. Redistilled water in not paraffined hard glass.
3. Distilled water " " " "

that it is safe to assume that the grains of *Oryza* germinate best in pure distilled water.

As already referred to, the grains of *Oryza* do not germinate even in solutions of considerably weak concentration, and also the subsequent growth of the plumule and the radicle is very much retarded by the presence of dissolved substances. One of the data obtained may serve to give an illustration.

TABLE XII. *Oryza sativa*.

Influence of concentration upon the development of shoots and roots. Average of 20 grains for 72 hrs. at 26° C.

	Shoot in mm.	Root in mm.
NaCl $\frac{1}{10}$ N	8.3	0.05
" $\frac{1}{50}$ N	16.95	0.4
" $\frac{1}{100}$ N	20.2	0.6
Cane sugar $\frac{1}{10}$ N	12.2	0.1
" $\frac{1}{20}$ N	14.4	0.5
" $\frac{1}{50}$ N	20.25	1.4
Redist. water	21.3	3.8

The retardation of growth seems due to an osmotic effect as well as a chemical one, for the effect of equimolecular solutions of a non-electrolyte exerts a similar influence to those of an electrolyte. Even grains which absorb as much water as is required for germination in distilled water, are yet unable to germinate in a normal cane sugar solution.

4. Tap water in not paraffined hard glass.
5.  $\text{CuSO}_4 \frac{1}{1000} \text{N}$  (ordinary distilled water used)
6. "  $\frac{1}{10000} \text{N}$  ( " " " )
7. "  $\frac{1}{100000} \text{N}$  ( " " " )
8.  $\text{ZnSO}_4 \frac{1}{500} \text{N}$  ( " " " )
9. "  $\frac{1}{1000} \text{N}$  ( " " " )
10. "  $\frac{1}{10000} \text{N}$  ( " " " )

## V. The Effect of Extremes of Temperature on the Germinative Power.

It is a well known fact that the seed can withstand extremes of temperature, especially in the desiccated condition. In regard to high temperature, HABERLANDT, as early as 1863, showed that the dried seed can withstand 48 hours' exposure at 100° C. JUST (1877) observed the increase in the resistance to heat by the degree of desiccation of the seed. VON HÖHNEL (1877) showed that fully dried seeds could withstand an hours' exposure at 110°—125° C. DIXON (1901) demonstrated that the seed of various kinds of plants, such as *Avena sativa*, *Lolium perenne*, *Lactuca sativa*, *Helianthus argophyllus*, *Mimulus moschatus*, *Medicago sativa*, *Brassica Rapa*, *Eschscholtzia californica*, *Papaver somniferum*, &c. can resist at least 100° C. The seed of *Medicago* can be germinated (10%) after one hours' exposure at 110° followed by another one hour's exposure at 121°. The effect of exposure to high temperature could in all cases be observed in the marked retardation of germination and in the extremely slow growth afterwards. WHITE (1909) was able to show that many of the seeds of barley, wheat, and oats could be germinated after  $\frac{1}{2}$  hours' exposure to 99—100° C, but after  $6\frac{1}{2}$  hours' exposure none of the seeds germinated. One hours' exposure to 122° C also destroyed the vitality of the seeds.

The effect of low temperature was investigated by DE CANDOLLE (1895), BROWN and ESCOMBE (1898), THISELTON-DYER (1899), BECQUEREL (1907) and WHITE (1909). BROWN and ESCOMBE exposed the seed of *Hordeum distichon*, *Avena sativa*, *Cucurbita Pepo*, *Cyclanthera explodens*, *Lotus Tetragonolobus*, *Pisum sativum*, *Trigonella Foenum-graecum*, *Impatiens balsamina*, *Helianthus annuus*, *Heracleum villosum*, *Convolvulus tricolor*, *Funkia Sieboldiana* to liquid air ( $-183^{\circ}$  to  $-192^{\circ}$  C) for 50 hours, but no appreciable difference was observed with the control seeds. The seeds of *Brassica alba*, *Pisum sativum*, *Cucurbita Pepo*, *Mimulus moschatus*, *Triticum sativum*, and *Hordeum vulgare* were tested by THISELTON-DYER. The subjection to extreme low temperature ( $-250^{\circ}$ ) "did not show the smallest visible trace of the ordeal." BECQUEREL (1907), showed that even if the seed coat is removed and the liquid air is allowed to penetrate into the embryo, no appreciable difference in the germi-

nating power is observed after exposure to  $-190^{\circ}\text{C}$  for 130 hours. Injury took place, however, if the seed was not desiccated.

WHITE (1909) subjected the seeds of wheat, barley, oats, rye and maize to liquid air for fully two days and found that these seeds were not practically affected in the power of germination. She found also that the enzymes contained in the seeds are not at all affected. The enzymes present within the resting seeds of the five different genera of cereals employed are not destroyed when thoroughly dried seeds are subjected to the extraordinarily wide range of temperature of  $-200^{\circ}\text{C}$  to  $+120^{\circ}\text{C}$ , i.e. a range of  $320^{\circ}\text{C}$ .

The following experiments were made: The desiccated and air-dried grains of *Oryza* and *Zea* were exposed to  $97^{\circ}-98^{\circ}\text{C}$  for two hours, and the germinative power was tested.

The grains of *Oryza*, *Zea* and the seeds of *Fagopyrum* were put in small muslin bags and allowed to stay in liquid air, which was kept in a vacuum jacketed tube wrapped in cotton rugs. The length of time of exposure to the low temperature was not certain, for the liquid air was found to be evaporated at the time of the last examination, but could not have been less than six hours. The results of the experiments are given in the following table.

TABLE XIII. The effect of extremes of temperature.

Seed	Condition	Percentage of germination		
		After liquid air	After high temp.	Control
<i>Zea Mays</i> , white Dent.	desiccated	92.8	0	100
" " "	air-dried	85.0	0	100
" " yellow Dent.	desiccated	100.0	0	100
" " "	air-dried	91.6	0	100
" " blue Flint	"	100.0	0	100
<i>Fagopyrum esculentum</i>	"	90.0	—*	90
<i>Oryza sativa</i> " Chiba-nishiki "	unhulled, desiccated	90.0	—	100
" " "	hulled air-dried	100.0	—	100

\* The experiment not performed.

Seed	Condition	Percentage of Germination		
		After liquid air	After high temp.	Control
<i>Oryza sativa</i> "Araki"	hulled, desiccated	100.0	—	100
" " " Aka-gome "	unhulled, desiccated	88.1	94.1	100
" " " "	hulled, desiccated	100.0	94.3	100
" " " Araki "	unhulled, air-dried	—	0	—
" " " Chiba-nishiki "	hulled, desiccated	—	97.1	—

As is shown above, the grain of *Oryza* is much more resistant than that of *Zea* to extremes of temperature, especially to high temperature, at least in the materials used.

## VI. Summary.

In the seed covering of *Oryza sativa* and of *Zea Mays* selective permeability is observed. The seat of the selective-permeable septum in the *Oryza* grain is most probably confined to the cutinized inner wall of the inner integument which lies directly above the aleurone layer in the fully matured grain.

The germinative power of the desiccated hulled grain of *Oryza* is slightly affected by twenty-four hours' steeping in 6 N sulphuric acid, chloroform, acetone, ethyl ether, commercial absolute ethyl alcohol, picric acid (aqueous solution), and the ethyl alcoholic (commercial absolute) solution of thymol, naphthalene and  $\alpha$ -naphthol, whereas the air-dried grains are killed by similar treatment. Likewise in the case with *Zea*, 5 N sulphuric acid, hydrochloric acid (twenty one hours), commercial absolute ethyl alcohol, and the ethyl alcoholic (commercial absolute) solution of naphthalene, resorcin,  $\alpha$ -naphthol, and  $\alpha$ -naphthylamine destroy the vitality of air-dried grains, but not the desiccated ones.

The vitality of the desiccated grains of *Oryza* (hulled) and *Zea* is lost by twenty-four hours' steeping in formaldehyde, formic acid, commercial absolute methyl alcohol, methyl ether, acetaldehyde, glacial acetic acid, butyric acid, amyl alcohol, pyridine, and the aqueous solution of chloralhydrate, resorcin hydroquinone and twenty one hours' steeping in nitric acid (3 N, 6 N).

Even the embryonal halves of the desiccated hulled grains of *Oryza* are capable of germination after twenty-four hours steeping in commercial absolute ethyl alcohol, ethyl ether, the ethyl alcoholic (commercial absolute) solution of resorcin, acetic acid, hydroquinone and naphthalene, but the air-dried entire hulled grains are killed by the similar treatments.

Twenty-four hours steeping in the aqueous solution of phenol, resorcin,  $\alpha$ -naphthol, hydroquinone, acetic acid and mercuric chloride is fatal to the desiccated and air-dried grains of *Oryza* (hulled) and *Zea*, whereas the corresponding alcoholic (commercial absolute) or ether solutions are harmful only to a considerable extent.

The hulled grain of *Oryza* can be germinated at an extreme low oxygen pressure, but under such condition the development of the radicle is totally prohibited. A supply of oxygen initiates the development of the radicle in the seedlings thus germinated.

No appreciable stimulating influence of H and OH ions is observed in the germination of the *Oryza* grains.

The germinative power of the grains of *Oryza*, *Zea* and the seeds of *Fagopyrum* is practically unaffected by a few hours' exposure to an extreme low temperature by means of steeping them in liquid air.

By two hours' exposure to 97—98°C, the germinative power of the grains of *Zea* is lost, but that of the grains of *Oryza*, especially if desiccated, is only slightly affected.

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December 1915.

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## EXPLANATION OF PLATE IX.

Figs. 1-2. *Oryza sativa*. Photomicrographs of the section of milk-ripened grain 1. Transverse section,  $\times 320$ . 2. Longitudinal section,  $\times 320$ . *p*=parenchym of pericarp, *t*=tube cells, *i*=integument, *c*=cutinized layer, *n*=nucellus, *a*=aleurone layer, *ch*=chlorophyll bodies.

Figs. 3-4. *Zea Mays*. Photomicrographs of the transverse section of milk-ripened grain. 3.  $\times 350$ . 4.  $\times 45$ . *p*=pericarp, *c*=cutinized layer, *a*=aleurone layer, *s*=starchy endosperm tissue.

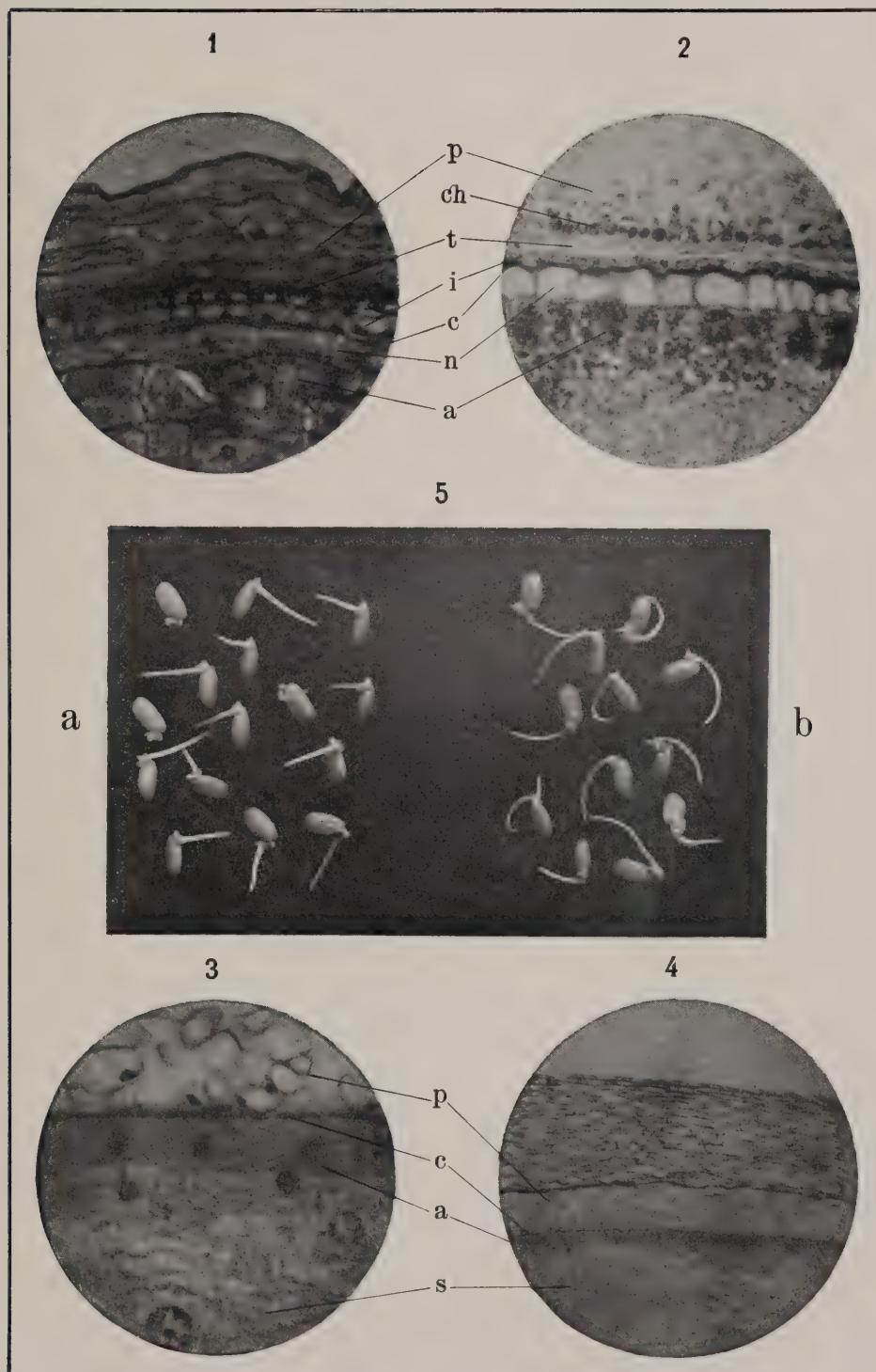
Fig. 5. *Oryza sativa* seedlings. *a*, germinated in the oxygen free air, showing the absence of the radicle. *b*, germinated in normal air, showing the presence of the radicle and the plumule.

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All communications relating to this Journal should be addressed to the Director of the College of Agriculture.

# Über die Serodiagnose der Schwangerschaft bei Pferden und Kühen mittelst des Abder- halden'schen Dialysierverfahrens.

VON

**Torai Shimamura und Shigeo Matsuba.**

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Seitdem der Hallenser Physiolog E. ABDERHALDEN eine Methode, die es ermöglicht, aus dem Blute die Schwangerschaft zu diagnostizieren, ausarbeitete, und im Jahre 1912 seine unermüdliche, langjährige Untersuchung veröffentlichte, wurde die Prüfung derselben von weit über 150 Autoren experimentell an Menschen gemacht. Die Meinungen über die Angaben ABDERHALDENS sind zur Zeit noch geteilt. Neben denjenigen, die in zahlreichen Fällen vorzügliche Resultate bekamen, erheben sich Stimmen, die die differentialdiagnostische Verwendbarkeit der ABDERHALDENSchen Reaktion anzweifeln. Während hier in der Humanmedizin eine so grosse Zahl von Versuchen vorliegt, ist das Problem in der Veterinärmedizin noch wenig in Angriff genommen worden. Aber gerade für unsere Wissenschaft hat die Trächtigkeitsdiagnose noch grössere praktische Bedeutung, als für die Humanmedizin. Aus diesem Grunde hat uns unser hochverehrter Lehrer, Herr Prof. Dr. G. SUTO, damit beauftragt, zu prüfen, ob die ABDERHALDENSche Methode auch bei den Haustieren ausführbar ist, und ob durch dieses Verfahren eine Frühdiagnose der Trächtigkeit erbracht werden kann. Wir haben von Anfang September 1913 bis Ende April 1914 die Technik der Methode (Dialysierverfahren) geübt und im Sommer 1914 in dem kaiserlichen Hofgestüt zu SHIMOSA Versuche mit den von ABDERHALDEN in No. 9 der Münchener medizinischen Wochenschrift 1912 angegebenen verschärften Vorschriften angestellt.

Es standen uns im ganzen über 75 Fälle, darunter 34 Kühe und 41 Pferde, zur Verfügung, abgesehen von zahlreichen Kontrollversuchen.

Die zu unseren Untersuchungen benutzten Tiere waren zum grössten Teil nur durch die äusseren Schwangerschaftszeichen, namentlich Ausbleiben der Brunst, Änderungen im Benehmen des Tieres, Auftriebung des Bauches, Anschwellen des Euters u. s. w. als trächtig bezeichnet worden. Diese äusseren Schwangerschaftszeichen sind wohl Anhalte, durch die man auf Trächtigkeit schliessen kann, aber eine sichere klinische Methode zur Bestimmung der Trächtigkeit besteht noch nicht. Unter unseren Versuchstieren gab es auch viele, die durch die mangelhafte klinische Methode als nichttragend diagnostiziert waren. Wir möchten diese klinische Diagnose mit dem Ergebnisse der serologischen Untersuchung nicht vergleichen, weil man dadurch allein nicht unterscheiden kann, welche Diagnose richtig ist, die klinische oder die serologische, wenn die Resultate des Dialysierverfahrens nicht mit der klinischen Diagnose übereinstimmen. Man kann nur erst nach der Geburt wissen, ob die klinische Schwangerschaftsdiagnose richtig war oder nicht. Aus diesem Grunde warteten wir über ein Jahr, während welcher Zeit die ganze Reihe der Versuchstiere ihre Schwangerschaftszeit durchlaufen konnte. Man kann aus den folgenden Tabellen ersehen, bis zu welchem Grade die klinische Diagnose mit dem Resultate der serologischen Untersuchung übereinstimmt, namentlich wie weit sich die Verwendbarkeit des ABDERHALDENschen Dialysierverfahrens zu rein klinischen Zwecken erstreckt.

TABELLE I.\*

## Versuche an Kühen.

Tiername.	Trächt. (Tage)	Versuchsreihe.	Ninhydrinreakt.	Sero-diagnose.	Bemerkungen.
Beauty II	8	Glas I 1.5 c.c. Serum + Plazenta	++		
		„ II „ „ + „	-	zweifelhaft	nichtträchtig
		„ III „ „ allein	++		
Dinah IV	11	Glas I 1.5 c.c. Serum + Plazenta	(+)		
		„ II „ „ + „	(+)	trächtig	Partus 12/V '15
		„ III „ „ allein	-		

\* ++ Starkviolette Färbung der Ninhydrinreaktion, + + mittelviolett, + schwachviolett, (+) Spur von Violettfärbung und - negativ.

Tiername.	Tricht. (Tage)	Versuchsreihe.	Ninhydrinreakt.	Sero-diagnose.	Bemerkungen.
Palace	14	Glas I 1.5 c.c. Serum + Plazenta „ II „ „ + „, „ III „ „ allein	++ + -	trächtig	Partus, aber der Geburtstag unklar
Success III	17	Glas I 1.5 c.c. Serum + Plazenta „ II „ „ + „, „ III „ „ allein	++ + -	trächtig	nichtträchtig
Lucky III	20	Glas I 1.5 c.c. Serum + Plazenta „ II „ „ + „, „ III „ „ allein	++ ++ -	trächtig	Verkauft 10/X '15
Weal II	21	Glas I 1.5 c.c. Serum + Plazenta „ II „ „ + „, „ III „ „ allein	++ + -	trächtig	Verkauft 20/X '15 damals zeigte das klinische Bild deutlich schwangerschaft.
Olympia	24	Glas I 1.5 c.c. Serum + Plazenta „ II „ „ + „, „ III „ „ allein	++ + -	trächtig	nichtträchtig
Wakefield II	24	Glas I 1.5 c.c. Serum + Plazenta „ II „ „ + „, „ III „ „ allein	(+) (+) -	trächtig	Partus, aber der Geburtstag nicht berichtet
Holy Head	26	Glas I 1.5 c.c. Serum + Plazenta „ II „ „ + „, „ III „ „ allein	++ ++ -	trächtig	Partus 26/VI '15
Bless II	27	Glas I 1.5 c.c. Serum + Plazenta „ II „ „ + „, „ III „ „ allein	++ + -	trächtig	Partus 22/I '15
Bless V	51	Glas I 1.5 c.c. Serum + Plazenta „ II „ „ + „, „ III „ „ allein	- (+) -	trächtig	nichtträchtig
Amalia II	56	Glas I 1.5 c.c. Serum + Plazenta „ II „ „ + „, „ III „ „ allein	+	trächtig	Partus 15/III '15
Sweet Secret	110	Glas I 1.5 c.c. Serum + Plazenta „ II „ „ + „, „ III „ „ allein	++ ++ -	trächtig	Partus 8/I '15

Tiername.	Trächt. (Tage)	Versuchsreihe.	Ninhydrinreakt.	Sero-diagnose.	Bemerkungen.
Cleanor	112	Glas I 1.5 c.c. Serum + Plazenta	(+)		
		„ II „ „ + „	++		
		„ III „ „ + „	(+)	trächtig	nichtträchtig
		„ IV „ „ allein	(+)		
Beauty	129	Glas I 1.5 c.c. Serum + Plazenta	(+)		
		„ II „ „ + „	(+)	trächtig	Partus 11/I '15
		„ III „ „ allein	-		
Bona II	132	Glas I 1.5 c.c. Serum + Plazenta	++		
		„ II „ „ + „	++	trächtig	Partus 18/XII '14
		„ III „ „ allein	-		
Weltje III	133	Glas I 1.5 c.c. Serum + Plazenta	++		
		„ II „ „ + „	++	trächtig	Partus 19/XII '14
		„ III „ „ allein	-		
Ruth II	145	Glas I 1.5 c.c. Serum + Plazenta	++		
		„ II „ „ allein	(+)	trächtig	Partus 28/XI '14
Amaryllis	151	Glas I 1.5 c.c. Serum + Plazenta	+		
		„ II „ „ + „	+	trächtig	Partus 24/XI '14
		„ III „ „ allein	-		
Lady's maid	152	Glas I 1.5 c.c. Serum + Plazenta	+		
		„ II „ „ + „	++	trächtig	Partus 5/XII '14
		„ III „ „ allein	-		
Dinah II	162	Glas I 1.5 c.c. Serum + Plazenta	+		
		„ II „ „ + „	++	trächtig	Partus 20/XI '14
		„ III „ „ allein	-		
Eche	184	Glas I 1.5 c.c. Serum + Plazenta	+		
		„ II „ „ + „	+	trächtig	Partus 24/X '14
		„ III „ „ allein	-		
Snowflake	193	Glas I 1.5 c.c. Serum + Plazenta	++		
		„ II „ „ + „	++	trächtig	Partus 17/X '14
		„ III „ „ + allein	-		
Amalia	211	Glas I 1.5 c.c. Serum + Plazenta	++		
		„ II „ „ + „	++	trächtig	Partus 1/X '14
		„ III „ „ allein	-		

Tiername.	Trächt. (Tage)	Versuchsreihe.	Ninhydrinreakt.	Sero-diagnose.	Bemerkungen.
Adema	215	Glas I 1.5 c.c. Serum + Plazenta	-		
		„ II „ „ + „	(+)?	nicht-trächtig	Partus 4/X '14
		„ III „ „ allein	-		
Dinah	216	Glas I 1.5 c.c. Serum + Plazenta	++		
		„ II „ „ + „	+	trächtig	Partus 20/X '14
		„ III „ „ allein	-		
Joyful	228	Glas I 1.5 c.c. Serum + Plazenta	++		
		„ II „ „ + „	++	trächtig	Partus 7/IX '14
		„ III „ „ allein	-		
Alurica III	238	Glas I 1.5 c.c. Serum + Plazenta	++		
		„ II „ „ + „	++	trächtig	Partus 9/IX '14
		„ III „ „ allein	-		
Family	259	Glas I 1.5 c.c. Serum + Plazenta	+		
		„ II „ „ + „	++	trächtig	Partus 14/VIII '14
		„ III „ „ allein	-		
Star II	261	Glas I 1.5 c.c. Serum + Plazenta	-		
		„ II „ „ + „	+	trächtig	Partus 6/VIII '14
		„ III „ „ allein	-		
Pembroke	264	Glas I 1.5 c.c. Serum + Plazenta	++		
		„ II „ „ + „	++	trächtig	Partus 19/VIII '14
		„ III „ „ allein	-		
Model maid	279	Glas I 1.5 c.c. Serum + Plazenta	+		
		„ II „ „ + „	+	trächtig	Partus 25/VII '14
		„ III „ „ allein	-		
Sarah	10 Tage nach der Geburt	Glas I 1.5 c.c. Serum + Plazenta	++		
		„ II „ „ + „	++		
		„ III „ „ allein	++		
Nellie II	nicht- trächtig	Glas I 1.5 c.c. Serum + Plazenta	-		
		„ II „ „ + „	(+)		
		„ III „ „ allein	-		
		„ IV „ „ „	-		
		„ V „ „ „	-		

Geschlachtet,  
nicht-trächtig

TABELLE II.\*

## Versuche an Stuten.

Tiername.	Trächt. (Tage)	Versuchsreihe.	Ninhydrinreakt.	Sero-diagnose.	Ammerkung.	Partus.
Grand Merit III	28	Glas I Serum 1.5 c.c.+Plazenta	-			
		" II " " allein	+G	nicht-trächtig	Bei Erhitzung das Reagenz-Glas IV gebrochen	nicht-trächtig
		" III " 1.0 c.c.+Plazenta	-			
		" IV " " allein	-			
Beauty II	34	Glas I Serum 1.5 c.c.+Plazenta	++G			
		" II " " allein	++G	trächtig	Die Farbenintensität von Glas I ist stärker als die von Glas II	2/vi '15
		" III " 1.0 c.c.+Plazenta	(+) G			
		" IV " " allein	-			
Orchid	35	Glas I Serum 1.5 c.c.+Plazenta	+			
		" II " " allein	-			26/v '15
		" III " 1.0 c.c.+Plazenta	(+) G	"		
		" IV " " allein	-			
Kachikumo	35	Glas I Serum 1.5 c.c.+Plazenta	(+) G			
		" II " " allein	(+) G	nicht-trächtig		9/vi '15
		" III " 1.0 c.c.+Plazenta	(+) G			
		" IV " " allein	(+) G			
Brenda	36	Glas I Serum 1.5 c.c.+Plazenta	+			
		" II " " allein	++	trächtig		8/vi '15
		" III " 1.0 c.c.+Plazenta	+	?		
		" IV " " allein	(+)			
Luck III	39	Glas I Serum 1.5 c.c.+Plazenta	+G			
		" II " " allein	+G	nicht-trächtig		nicht-trächtig
		" III " 1.0 c.c.+Plazenta	-			
		" IV " " allein	-			
Bustle	42	Glas I Serum 1.5 c.c.+Plazenta	++			
		" II " " allein	-	trächtig		nicht-trächtig
		" III " 1.0 c.c.+Plazenta	-			
		" IV " " allein	-			

\* Bezeichnung der Farbenintensität der Ninhydrinreaktion ist wie folgt: — + starkviolett, ++ mittelviolet, + schwachviolett, (+) Spur von Violettfärbung, — negativ. Der Buchstabe G bedeutet gelblichen Ton, R rötlichen und (G) Spur von gelblichem Tone, z. B. ++(G) bedeutet Mittelviolettfärbung mit Spur von gelblichem Tone.

ÜBER DIE SERODIAGNOSE DER SCHWANGERSCHAFT BEI PFERDEN, ETC. 165

Tiername.	Trächt. (Tage)	Versuchsreihe.	Ninhydrinreakt.	Sero-diagnose.	Ammerkung.	Partus.
Carbrooke	43	Glas I Serum 1.5 c.c.+Plazenta	++			
		" II " " allein	-			
		" III " 1.0 c.c.+Plazenta	(+) G	trächtig		8/vi '15
		" IV " " allein	--			
Golden Rod II	44	Glas I Serum 1.5 c.c.+Plazenta	(+) G			
		" II " " allein	+G			
		" III " 1.0 c.c.+Plazenta	(G)	nicht-trächtig		28/v '15
		" IV " " allein	(G)			
Brownie	48	Glas I Serum 1.5 c.c.+Plazenta	+G			
		" II " " allein	(+) R			
		" III " 1.0 c.c.+Plazenta	-	trächtig		19/v '15
		" IV " " allein	-			
Wealth II	50	Glas I Serum 1.5 c.c.+Plazenta	(+) G			
		" II " " allein	(+) G			
		" III " 1.0 c.c.+Plazenta	(+) G	"	Die Farben-Intensität von Glas III ist stärker als die von Glas II	28/v '15
		" IV " " allein	-			
Brenda III	54	Glas I Serum 1.0 c.c.+Plazenta	++			
		" II " " allein	+G			
		" III " 1.5 c.c.+Plazenta	(+) G	"		6/v '15
		" IV " " allein	-			
Pansy VII	55	Glas I Serum 1.5 c.c.+Plazenta	-			
		" II " 1.0 c.c. " "	-	nicht-trächtig		
		" III " 1.5 c.c. allein	-			nicht-trächtig
Prosperity	57	Glas I Serum 1.5 c.c.+Plazenta	++			
		" II " " allein	+			
		" III " 1.0 c.c.+Plazenta	(+)	trächtig		2/vi '15
		" IV " " allein	-			
Parade IV	64	Glas I Serum 1.5 c.c.+Plazenta	++			
		" II " 1.0 c.c. " "	++	"		
		" III " 1.5 c.c. allein	-			nicht-trächtig
Butter Cup	78	Glas I Serum 1.5 c.c.+Plazenta	+G			
		" II " " allein	(+)		Glas III ist von stärkerer Färbung als Glas II	
		" III " 1.0 c.c.+Plazenta	(+)	"		22/vi '15
		" IV " " allein	-			

Tiername.	Trächt. (Tage)	Versuchsreihe.	Ninhydrinreakt.	Sero-diagnose.	Ammerkung.	Partus.
Carmen	81	Glas I Serum 1.5 c.c.+Plazenta	++			
		„ II „ „ allein	(+) G			
		„ III „ 1.0 c.c.+Plazenta	—	trächtig		8/V '15
		„ IV „ „ allein	—			
Catherina	83	Glas I Serum 1.5 c.c.+Plazenta	—			
		„ II „ „ allein	—			
		„ III „ 1.0 c.c.+Plazenta	—	nicht-trächtig		4/IV '15
		„ IV „ „ allein	—			
Laurel IV	89	Glas I Serum 1.5 c.c.+Plazenta	—			
		„ II „ „ allein	(+) G			
		„ III „ 1.0 c.c.+Plazenta	(+) G	”		19/IV '15
		„ IV „ „ allein	—			
Better Still	92	Glas I Serum 1.5 c.c.+Plazenta	++			
		„ II „ „ allein	(+) G			
		„ III „ 1.0 c.c.+Plazenta	(+) G	trächtig	Die Farbenintensität von Glas III ist stärker als die von Glas II	nicht-trächtig
		„ IV „ „ allein	+G			
Happiness VI	98	Glas I Serum 1.5 c.c.+Plazenta	++			
		„ II „ „ allein	+G			
		„ III „ 1.0 c.c.+Plazenta	(+)	”		27/III '15
		„ IV „ „ allein	(+)			
Captivation III	99	Glas I Serum 1.5 c.c.+Plazenta	+G			
		„ II „ „ allein	+G			
		„ III „ 1.0 c.c.+Plazenta	(+) G	”		nicht-trächtig
		„ IV „ „ allein	—			
Caprice	100	Glas I Serum 1.5 c.c.+Plazenta	++			
		„ II „ „ allein	+			
		„ III „ 1.0 c.c.+Plazenta	(+)	”		Abortus 18/XII '14
		„ IV „ „ allein	—			
Belladonna	101	Glas I Serum 1.5 c.c.+Plazenta	++G			
		„ II „ 1.2 c.c. „ „	(+) G	nicht-trächtig		
		„ III „ 1.5 c.c. allein	++G			nicht-trächtig
Ishiyi	104	Glas I Serum 1.5 c.c.+Plazenta	(+) G			
		„ II „ „ allein	(+) G			
		„ III „ 1.0 c.c.+Plazenta	—	”		
		„ IV „ „ allein	—			nicht-trächtig

ÜBER DIE SERODIAGNOSE DER SCHWANGERSCHAFT BEI PFERDEN, ETC. 167

Tiername.	Trächt. (Tage)	Versuchsreihe.	Ninhydrinreakt.	Sero-diagnose.	Ammerkung.	Partus.
Beacon	105	Glas I Serum 1.5 c.c.+Plazenta	(+) G			
		" II " " allein	++	zweifelhaft		5/IV '15
		" III " 1.0 c.c.+Plazenta	(+) G			
		" IV " " allein	(+) G			
Bay Thorpe	108	Glas I Serum 1.5 c.c.+Plazenta	+G			
		" II " " allein	+G	nicht-trächtig		nicht-trächtig
		" III " 1.0 c.c.+Plazenta	-			
		" IV " " allein	-			
Cilnius	109	Glas I Serum 1.5 c.c.+Plazenta	(+) G			
		" II " " allein	(+) G	"		"
		" III " 1.0 c.c.+Plazenta	(+) G			
		" IV " " allein	(+) G			
Castalis	112	Glas I Serum 1.5 c.c.+Plazenta	(+) G			
		" II " " allein	(+) G	"		"
		" III " 1.0 c.c.+Plazenta	-			
		" IV " " allein	(+) G			
Music IV	114	Glas I Serum 1.5 c.c.+Plazenta	++	trächtig	Die Farbenintensität von Glas I ist stärker als die von Glas II	21/III '15
		" II " " allein	++			
		" III " 1.0 c.c.+Plazenta	+			
		" IV " " allein	-			
Parade	115	Glas I Serum 1.5 c.c.+Plazenta	+G	nicht-trächtig		28/III '15
		" II " " allein	+G			
		" III " 1.0 c.c.+Plazenta	(+) G			
		" IV " " allein	(+) G			
Happiness	116	Glas I Serum 1.5 c.c.+Plazenta	++G	trächtig?		1/IV '15
		" II " " allein	+G			
		" III " 1.0 c.c.+Plazenta	-			
		" IV " " allein	(+) G			
Accolte	117	Glas I Serum 1.5 c.c.+Plazenta	+G	trächtig		5/IV '15
		" II " " allein	+G			
		" III " 1.0 c.c.+Plazenta	++			
		" IV " " allein	+G			
Caroline	121	Glas I Serum 1.5 c.c.+Plazenta	++	"	Glas I ist von stärkerer Farbenintensität als Glas II	12/III '15
		" II " " allein	++			
		" III " 1.0 c.c.+Plazenta	++			
		" IV " " allein	-			

Tiername.	Trächt. (Tage)	Versuchsreihe.	Ninhydrinreakt.	Sero-diagnose.	Ammerkung.	Paruts.
Bonta	128	Glas I Serum 1.5 c.c. + Plazenta	++			
		„ II „ „ allein	+			
		„ III „ 1.0 c.c. + Plazenta	(+)	trächtig		12/III '15
		„ IV „ „ allein	-			
Bow Bell	129	Glas I Serum 1.5 c.c.+Plazenta	++			
		„ II „ „ allein	++			
		„ III „ 1.0 c.c.+Plazenta	++	nicht-trächtig?		8/III '15
		„ IV „ 0.2 c.c. allein	-			
Spring field IV	132	Glas I Serum 1.5 c.c.+Plazenta	++			
		„ II „ 1.0 c.c. „ „	+	trächtig		14/II '15
		„ III „ 1.5 c.c. allein	(+)			
Calinus	139	Glas I Serum 1.5 c.c.+Plazenta	+G			
		„ II „ 1.0 c.c. „ „	+G		„	22/II '15
		„ III „ 1.5 c.c. allein	(+) G			
Glory III	141	Glas I Serum 1.5 c.c.+Plazenta	(+) G			
		„ II „ 1.0 c.c. „ „	+G		„	7/III '15
		„ III „ 1.5 c.c. allein	-			
Honey suckle	143	Glas I Serum 1.5 c.c.+Plazenta	(+) G			
		„ II „ 1.0 c.c. „ „	+G		„	22/II '15
		„ III „ 1.5 c.c. allein	-			
Captivation III	147	Glas I Serum 1.5 c.c.+Plazenta	(+) G		Glas I & III sind von stärkerer Färbung als Glas II	
		„ II „ 1.0 c.c. „ „	(+) G	trächtig?		
		„ III „ 1.5 c.c. allein	(+) G			11/II '15

Wie aus den oben beschriebenen Tabellen ersichtlich, haben wir beim Rinde unter 34 Versuchen 28 mal = 81%, beim Pferde unter 41 Köpfen 31 mal = 75% richtige Resultate.

#### ZUSAMMENFASSUNG.

1. Die ABDERHALDENsche Methode (das Dialysierverfahren) kann auch bei unseren Haustieren (Kühen, Stuten) zur Diagnosestellung der Schwangerschaft angewendet werden.
2. Mit Hilfe dieses Verfahrens lässt sich die Schwangerschaftsdiagnose erbringen, doch gibt es auch Fälle, wo die Reaktion versagt.

3. Meistens zeigt die Ninhydrinprobe bei Kühen einen tieferen Farbenton als bei Stuten.

4. Aus der Farbenintensität der Ninhydrinreaktion lässt sich nicht schliessen, wie lange Zeit das betreffende Tier tragend ist.

5. In unseren Versuchen war es beim Rinde schon 10 Tage und beim Pferde 34 Tage nach erfolgter Konzeption möglich, mittelst dieses Verfahrens den Trächtigkeitsnachweis zu erbringen.

6. Beim Rinde fällt die Ninhydrinreaktion zur Geburtszeit, ja sogar 10 Tage nach der Geburt noch positiv aus. Wir hatten damals hochtragende Stuten leider nicht zur Verfügung.

7. Der Trächtigkeitsnachweis ist bei Stuten bedeutend schwieriger zu führen als bei Kühen.

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# **Mallophaga from Birds of Formosa.**

By

**Seinosuke Uchida.**

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With Plate X and One Text-Figure.

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The descriptions of new, and determinations of old, species of Mallophaga, presented in this paper are based on the first collection that has ever been made of specimens of this ecto-parasitic insect found on Formosan birds. This small but interesting collection of Mallophaga is composed of specimens which were taken by Mr. NAGAMICHI KURODA, research scholar on ornithology, Tokyo Imperial University, during the spring of this year (1916), from the host specimens shot by himself. Mr. KURODA was kind enough to give this collection of parasites to the writer. The identification of the birds themselves, however, is his own.

The collection includes 8 genera and 21 species out of 13 host species of birds. Of the above number, 7 seem to me to be new to science and are here described for the first time. In addition, one new variety is recognized.

The list of host species with their parasites is given below:

<i>Nannocnus cinnamomea.</i> ....	<i>Laemobothrium loomisi</i> Kellogg & Chapman.
<i>Accipiter virgatus.</i> .....	<i>Lipeurus variabilis</i> Nitzsch. <i>Nirmus vittatus</i> Giebel.
<i>Milvus ater govinda.</i> .....	<i>Colpocephalum osborni</i> Kellogg.
<i>Pandion haliaetus.</i> .....	<i>Colpocephalum pachygaster</i> Giebel.
* <i>Calophasia mikado.</i> .....	<i>Nirmus ovatus</i> n. sp. <i>Lipeurus variabilis</i> Nitzsch. <i>Lipeurus intermedius</i> var. <i>major</i> n. var. <i>Goniodes intermedius</i> Neumann. <i>Menopon productum</i> Piaget. <i>Menopon mikadokijii</i> n. sp.

	<i>Lipeurus formosanus</i> n. sp.
	<i>Lipeurus rubrifasciatus</i> Piaget.
* <i>Arboricola crudigularis</i> .....	<i>Goniocotes microcephalus</i> n. sp.
	<i>Menopon pallescens</i> Nitzsch.
	<i>Menopon longipectum</i> n. sp.
<i>Tringa subminuta</i> .....	<i>Colpocephalum umbrinum</i> var. <i>trilobatum</i> Giebel.
<i>Tringa ruficollis</i> . ....	<i>Nirmus incænis</i> Kellogg & Chapman.
<i>Turtur chinensis</i> .....	<i>Lipeurus baculus</i> Nitzsch.
<i>Sphenocercus sororius</i> .....	<i>Lipeurus baculus</i> Nitzsch. <i>Goniocotes kurodai</i> n. sp.
<i>Pericrocotus griseigularis</i> . ..	<i>Docophorus communis</i> Nitzsch.
<i>Grauculus rex-pineti</i> .....	<i>Lipeurus baculus</i> Nitzsch. <i>Goniocotes kurodai</i> n. sp.
* <i>Urocissa cœrulea</i> .....	<i>Menopon urocissæ</i> n. sp.

Of the thirteen bird species, forming the list of the hosts, three, marked with asterisks, are species peculiar to this Island and many of the parasites found on them are new species.

The mikado pheasant, *Calophasis mikado* of Mt. Arisan, the most interesting species of all Formosan birds, is parasitized by six Mallophagan species of which two are new species and the other four common to several other pheasants except one which differs so considerably from the type that it must be referred to as variety. From *Arboricola crudigularis* (Phasianidæ) which is found throughout the Island at higher altitudes, five Mallophagan species were obtained, of which three were new species and two were known species, i. e., *Menopon pallescens* Nitzsch and *Lipeurus rubrifasciatus* Piaget.

None of the Mallophagan parasites of Campephagidæ have hitherto been recorded. Of this family our list contains two species, i. e. *Pericrocotus griseigularis* and *Grauculus rex-pineti*, and the former was found to be parasitized by *Docophorus communis* Nitzsch, the most widely spread species among Passeriforme hosts. From the latter were taken two parasites, one of them being *Lipeurus baculus* common to doves and the other a new *Goniocotes* which was also found in the wedgetailed pigeon, *Sphenocercus sororius*. This unusual distribution may be due either to the fact that these two host species have a similar

habitat or it may be caused by the parasites straggling in the game-bag from one bird to another.

The present collection is too small, so until a greater number of hosts and of Mallophagan species are investigated, it is scarcely worth while to attempt any general remarks concerning the bird-infesting Mallophaga of this interesting Island.

It is my pleasant duty here to express my cordial thanks to Mr. NAGAMICHI KURODA whose kindness enabled me to examine not only the present collection, but also some of the important materials in my studies of Japanese Mallophaga.

Gen. *DOCOPHORUS* Nitzsch.

**1. *Docophorus communis* Nitzsch.**

Germar's Mag. f. Ent., 1818, III, p. 290; Kellogg, New Mallophaga II, 1896, p. 486.

Three females and four youngs were collected from *Pericrocotus griseigularis* at Suisha, Distr. Nantō, May 3.

Gen. *NIRMUS* Nitzsch.

**2. *Nirmus vittatus* Giebel.**

Giebel, Insecta Epizoa, 1874, p. 127; Piaget, Les Pediculines, 1880, p. 132, pl. xi, fig. 2; Waterston, Ann. South Afr. Mus. x, 1914, p. 288.

Three females of this species were collected from *Accipiter virgatus* at Shūshū, Distr. Nantō, May 4.

**3. *Nirmus incœnis* Kellogg and Chapman.**

Kellogg and Chapman, New Mallophaga, III, 1899, p. 81, pl. vi, fig. 5.

Four female specimens were taken from *Tringa ruficollis* at Enteishō, Distr. Tainan, May 9.

4. *Nirmus ovatus* n. sp.

(Pl. X, Fig. 3.)

Two specimens, both males, obtained from *Calophasis mikado* Mt. Arisan, May 2.

## Measurements.

	♂ mm.	♂ mm.
Length of body .....	1.50	1.50
Width of body .....	0.60	0.60
Length of head .....	0.49	0.49
Width of head .....	0.42	0.42
Length of thorax .....	0.27	0.26
Width of thorax.....	0.36	0.36
Antenna .....	0.24	0.24

*Description of Male* :—Body short and broad, sub-docophoroid in form; ground colour of body pale yellowish brown, showing brownish marginal markings.

Head somewhat conical; front broadly parabolic, with five rather long marginal hairs, three of which on the margin of clypeus, the other two hairs in front of the trabeculae; two dorsal hairs, one between first and second marginal hairs and the other between second and third; trabeculae large, conical, uncoloured. Antennae well developed, with the second segment longest and the fourth shortest; the basal segment much thickened and second in length, about equal to the last segment; colour of antennae a little paler than the head and the last two segments darker. Eyes clear, prominent, each with a long hair; temples wide behind the eye; temporal margins convex, converging posteriorly, each with two long hairs and two short spines, occipital margin straight with a prickle near each temporal angle. Colour of head pale yellowish in median region; frontal bands broad, yellowish brown, continuous around the front, sides darker; as usual the bands turn inward before the base of antennae, forming an oblong, dark brown antennal blotch on either side; ocular blotches (small, distinct, reddish brown; very narrow brownish marginal band completely encircles each of the temples; occipital band chestnut brown with two prominent, roundish blotches of the same colour.

Prothorax short, width greater than length, somewhat hexagonal; lateral

margins short, each with a coloured swelling and a long hair; posterior margin truncate; marginal bands irregular, brownish. Metathorax short, trapezoidal; lateral margins bare and diverging posteriorly; posterior angles rounded, each bearing a hair and a fine prickle; posterior margin convex, angulated in middle, with eight long pustulated hairs in four pairs; marginal bands broad, present only on the lateral borders. Legs paler than body, with brownish marginal markings and a few scattered hairs.

Abdomen oval, broadest at the fourth segment, posterior angles protruding, bearing one to three hairs on segments III-VIII. Dorsal surface of abdomen with four rows of short hairs in the median portion of segments I-VII, and one row of long hairs behind the spiracles on the posterior margin of segments II-VIII. Segments I-V approximately equal in length, then successively shorter; the last segment round, entire, with numerous hairs. Lateral marginal bands brownish, well chitinized, entering into the segment preceding and curving directly backward to the posterior margin of the same; transverse blotches, broad, pale yellowish brown, entirely across all segments, leaving uncoloured stigmatal spots and a narrow pale line along each posterior margin of segments. Genitalia distinct, reaching to the fifth abdominal segment, and the posterior end consists of a triangular median part and two short chitinous movable hooks.

Gen. *GONIOCOTES* Burmeister.

**5. *Goniocotes kurodai* n. sp.**

(Plate X, Fig 4.)

One male specimen was taken from *Sphenocercus sororius*, killed at Suisha, Distr. Nanto, May 3, and another from *Grauculus rex-pineti* shot at the same locality, May 4.

This new species somewhat resembles Rudow's *Goniocotes carpophagæ*\*, but may be distinguished from that species chiefly by its larger head and broader abdomen.

Measurements.

	♂ mm.	♀ mm.
Length of body .....	1.07	1.07
Width of body .....	0.63	0.61

\* In Taschenberg's Die Mallophagen, p. 99, Taf. II, Fig. 10, 10a.

	♂ mm.	♂ mm.
Length of head .....	0.41	0.41
Width of head .....	0.51	0.51
Length of thorax .....	0.19	0.19
Width of thorax.....	0.37	0.37
Antenna .....	0.13	0.13

*Description of male* :—Ground colour of head and thorax yellowish orange, with well-defined, chestnut-brown markings; abdomen very pale, curved marginal bands pale yellowish.

Head with laterally projecting temples; front very broad, convex, with five very fine marginal hairs on each side, the second from the most anterior being somewhat longer; trabeculae absent; antennae short; the first segment long, extending beyond antennal sinus, the second longest, the fifth longer than the third or the fourth which is shortest and both are about equal. Eyes inconspicuous, a fine prickle behind each eye on lateral margin; temples expand laterally, each forming a protuberance which has a long, stout hair; a little behind the hair, another long one present; posterior portion of the temples slightly extended and angulated, with a very fine prickle; occiput convex. Ground colour of head yellowish orange; frontal band reddish brown, broadest in the centre and with irregular internal border; just in front of each antenna, a long chestnut-brown antennal blotch, running backward from the end of the frontal band; along the posterior margin of the frontal band, a well-defined, transverse, semitransparent space; ocular blotches large, rounded, reddish brown; a broad, yellow band runs along the temple from the eye to the posterior angle; occipital band, distinct, reddish brown, with two chestnut brown occipital blotches.

Prothorox short, narrow, trapezoidal; almost entirely included between the two posterior angles of the head; lateral margins diverging posteriorly, the posterior margin almost straight; lateral posterior angles slightly projecting, each with a long hair; with broad, distinct reddish brown submarginal bands. Metathorax with rounded lateral angles, each bearing two long hairs; posterior margin convexly abutting on abdomen, with two weak hairs on each side nearer to lateral angle than to the middle. Each lateral band broad, reddish brown, bending inward along anterior margin and uniting with the prothoracic band. Legs short and stout, paler than thorax, with pale brownish marginal markings and some scattered spines.

Abdomen very short and broad, width greater than length; entire surface almost clear, except the lateral and transverse bands; posterior angles slightly protruding, bearing one hair on segments II-IV; two hairs on segments V and VI and four hairs on segment VII; the last segment broad, entire with four weak hairs on posterior margin. Dorsal surface of the abdomen with a hair on each side of segments V and VI, just above the lateral bands; and four short hairs on the median portion of segments II-V and two on segments VI and VII. Lateral marginal bands yellowish, well chitinized, entering into the segment preceding and curving inward; transverse blotches short, indistinct, pale yellowish. Genitalia slender with weakly chitinized rods reaching to the second abdominal segment.

### 6. *Goniocotes microcephalus* n. sp.

(Plate X, Fig. 1.)

Six females and two young individuals were taken from *Arboricola eruditigularis* shot on Mt. Arisan.

This form somewhat resembles *Goniocotes erygaster* Piaget from the same host species, and is distinguished from that species through difference in size, shape of abdomen, and in chaetotaxy of head and thorax.

Measurements of the female specimens on hand:

	♀ mm.	♀ mm.	♀ mm.	♀ mm.	♀ mm.	♀ mm.
Length of body .....	1.30	1.25	1.25	1.30	1.29	1.27
Width of body .....	0.69	0.66	0.66	0.69	0.68	0.67
Length of head .....	0.38	0.36	0.36	0.38	0.38	0.38
Width of head .....	0.45	0.44	0.43	0.45	0.44	0.44
Length of thorax .....	0.24	0.23	0.23	0.24	0.23	0.23
Width of thorax .....	0.38	0.37	0.37	0.38	0.38	0.38
Antenna .....	0.15	0.15	0.15	0.15	0.15	0.15

*Description of female*:—Ground colour of head and thorax pale yellowish, with yellowish marginal markings and reddish brown blotches; abdomen paler, marginal bands and transverse blotches yellowish.

Head somewhat resembles that of *Goniocotes megalcephalus* Uchida\*; front very broad, convex, with six fine hairs on each side, and a short prickle just before each antenna; trabeculae wanting; antennae short, the first

\* Annot. Zool. Jap. vol. IX, p. 88.

segment broad, as long as the second or fifth segment, the third and fourth segments about equal and half the length of the other segments. Eyes large, but indistinct, each with a short spine on its posterior margin; another short spine behind eye on lateral margin; temples rounded and more expanded than those of *G. megalcephalus*, bearing two long, strong, hairs and two very fine prickles; posterior parts slightly expanded, terminating in two rounded projections, each bearing a short prickle; occiput sinuous, occipital margin nearly straight. Dorsal surface of head with four short hairs, two shorter ones on the clypeus and the other two near the bases of antennæ. Ground colour of the head pale yellowish; frontal band yellow, terminating in reddish brown antennal blotch, in front of each antenna; temporal bands narrow, clear yellowish, broadening posteriorly; occipital band distinct, with two dark brown occipital blotches.

Prothorax short, narrow, trapezoidal; anterior margin concave; posterior margin slightly convex; lateral margins diverging posteriorly; with pale, indistinct submarginal bands; lateral angles slightly protruded, bearing a longish hair. Metathorax triangular, with apex forming an obtuse angle on the abdomen; anterior margin straight; lateral angles rounded, much protruded, each bears two long, pustulated hairs; and a short hair on the dorsal surface of each angle; anterior lateral margins with broad yellowish marginal bands which end in round, reddish brown blotches. Legs short, paler than the body, with a few spines.

Abdomen broadly elliptical; widest at third and fourth segments; the first segments longest at side but short in middle, due to the backward projecting angle of the thorax; other segments almost equal in length; posterior angles of segments not protruded, bearing one to three long hairs, except on the first segment; segment II with a hair, segments III and IV with two, and segments V-VII with three hairs; the last segment broadly rounded, with a slight emargination, bearing six long and several short hairs. Dorsal surface of the abdomen with two rows of short hairs on the median portion of segments I-VII and a row of long hairs behind each spiracle on segments III-VI. Ground colour of the abdomen pale yellowish; lateral marginal bands very narrow, clear yellowish; bending inward along the posterior margins of the preceding segment and ending in darker, rounded

blotches; transverse blotches yellow, narrower inward, much darker towards the lateral margins, with a clear space for the spiracles and leaving a broad whitish median space.

Gen. *GONIODES* Nitzsch.

**7. *Goniodes intermedius* Neumann.**

Neumann, Archives de Parasitologie, xv, 1913, p. 627, figs. 15-18.

Four males, two females, three young males and three young females were collected from *Calophasia mikado* from Mt. Arisan, May 11.

The present specimens agree well with Neumann's description and figures of those from Pukras pheasant *Pucrasia darwini* of Eastern China, except that in our specimens, the head of male is somewhat shorter and broader. Measurements of the specimens on hand are as follows:

	♂ mm.	♂ mm.	♂ mm.	♂ mm.	♀ mm.	♀ mm.
Length of body .....	2.70	2.60	2.50	2.60	3.10	3.20
Width of body .....	1.50	1.40	1.32	1.40	1.50	1.60
Length of head .....	0.70	0.70	0.70	0.71	0.83	0.84
Width of head .....	0.89	0.88	0.86	0.88	1.15	1.18
Length of thorax .....	0.62	0.62	0.61	0.62	0.63	0.63
Width of thorax.....	0.83	0.81	0.77	0.83	0.84	0.86
Antenna .....	0.56	—	—	0.55	0.28	0.28

Gen. *LIPEURUS* Nitzsch.

**8. *Lipeurus formosanus* n. sp.**

(Text-fig. 1.)

A single female specimen was obtained from *Arboricola crudigularis* shot on Mt. Arisan, May 14.

This species resembles Carriker's *L. postemarginatus*\*, but differs markedly from it in markings of head, size and shape of thorax and abdomen.

*Description of female* :—Body length 2 mm., width 0.42 mm.; ground colour of body pale yellowish, showing brownish blotches on head and thorax and yellowish marginal bands on abdomen.

\* University Studies, Nebraska, vol. VIII, 1903, p. 25, pl. III, fig. 4.

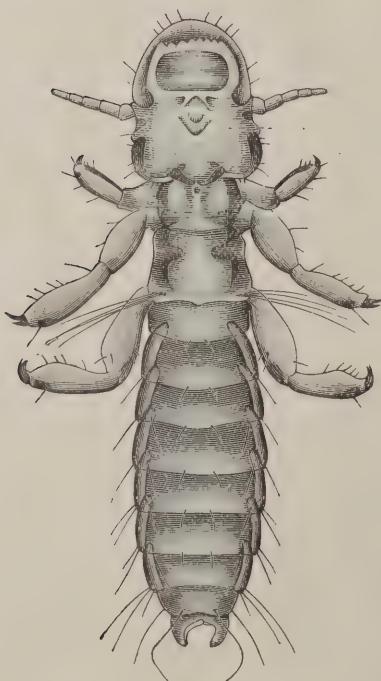
Head length 0.52 mm., width 0.40 mm.; somewhat conical; front broadly parabolic, with six fine hairs on each side, of which four being on the margin of clypeus and two in front of trabeculae; broad yellow marginal band completely encircling the front, broadest at clypeal part and showing distinct serrations on its inner margin; the lateral part of the band darker and the end bending inward in front of the base of each antenna and forming a chestnut brown antennal blotch; trabeculae very small, colourless. Antennae pale fulvous, the first segment broadest, subequal to the second or fifth segment, the third segment slightly shorter than the second and the fourth shorter than the third. Eyes distinct, colourless, each with a short hair; two spines behind eye on lateral margin of temples; temples rounded, slightly expanded, with one long, weak hair and a short spine near the angle, and

with a narrow brownish lateral band and around brownish blotch between eye and antenna; occipital margin with two short spines near each temporal angle; occiput W-shaped; occipital band clear brownish with two chestnut-brown occipital blotches.

Prothorax small, quadrilateral; length 0.14 mm., width 0.26 mm.; with lateral margins convex and slightly converging posteriorly; the anterior margin somewhat concave; the posterior margin straight; a short hair on each posterior angle. Lateral bands clear brownish with an indistinct chestnut-brown blotch near each posterior angle. Metathorax quadrilateral, length 0.23 mm., width 0.31 mm.; with anterior and posterior margins straight, lateral margins slightly concave in middle and somewhat diverging posteriorly; anterior angles much

Text-fig. 1. *Lipeurus formosanus*  
n. sp., female. ca.  $\times 42$ .

protruded antero-laterally, posterior angles distinct, each with a short spine and a short hair; four very long, stout hairs on each side of the posterior margins near posterior angles. Legs paler than body, with dark brown, dorsal



markings of femora and tibiæ.

Abdomen narrow, elongate, subparallel sided; widest at fourth and fifth segments; the first segment narrower and shorter than those that follow, with a large but indistinct brownish protuberance on each side; posterior angles of segments II-IV bearing one short hair; those of segments V and VI with one short and one long hair, those of segment VII with three very long hairs; the last segment conical, with the tip deeply emarginated, forming a kind of fork; and a very long hair on each lateral margin. Dorsal surface of the abdomen with two or four rows of hairs; two rows near the middle of posterior margins of segments I-IV, and additional two rows just inside of the broad yellowish lateral bands of segments III-VII.

### 9. *Lipeurus rubrifasciatus* Piaget.

(Plate X, figs. 6, 6a, 6b.)

Piaget, Les Pediculines, Supplément, 1885, p. 71, pl. VII, fig. 8.

Two males and a female were taken from *Arboricola crudigularis* shot on Mt. Arisan, May 4.

*Description of female* :—Body larger, temporal margins more convex than those of male; the second segment of antenna longest; the first and fifth segments slightly shorter and subequal, the third segment much shorter and the fourth segment shortest; segments of abdomen nearly equal in length; the eighth and ninth abdominal segments more distinctly divided than in the male; lateral margins of the eighth segment convex, bearing a series of fine hairs; emargination of the last segment narrower.

### 10. *Lipeurus variabilis* Nitzsch.

Denny, Manogr. Anopl. Brit., 1842, p. 164, pl. xv, fig. 6; Giebel, Insecta Epizoa, 1874, p. 219, Taf. XVI, fig. 3; Piaget, Les Pediculines, 1880, p. 364, pl. XXIX, fig. 4.

A female specimen was taken from *Calophasis mikado* from Mt. Arisan, May 11, and another male specimen obtained from *Accipiter virgatus* at Shûshû, Distr. Nantô, May 4. The latter was, of course, a case of straggler from a pheasant killed by the hawk.

**11. *Lipeurus intermedius* var. *major* n. var.**

Eight males, three females and numerous youngs were collected on *Calophasis mikado* from Mt. Arisan, May 11.

The present variety agrees closely with Piaget's description of the type species, but is uniformly larger.

Measurements of *Lipeurus intermedius major*.

	♀	♀	♀	♂	♂	♂	♂	♂	♂	♂	♂
	mm.										
Length of body.....	3.42	3.40	3.32	2.90	2.90	3.00	2.90	2.80	3.00	2.90	2.90
Width of body.....	0.87	0.87	0.87	0.66	0.66	0.60	0.63	0.64	0.63	0.63	0.66
Length of head.....	0.64	0.67	0.68	0.66	0.64	0.63	0.64	0.63	0.66	0.64	0.64
Width of head.....	0.45	0.45	0.45	0.42	0.41	0.42	0.42	0.40	0.41	0.40	0.40
Length of thorax....	0.64	0.63	0.63	0.59	0.60	0.60	0.60	0.60	0.62	0.60	0.60
Width of thorax ....	0.52	0.54	0.55	0.49	0.49	0.49	0.50	0.49	0.50	0.49	0.49
Antenna .....	0.35	0.35	—	—	—	—	—	0.50	—	—	—

**12. *Lepeurus baculus* Nitzsch.**

Denny, Monogr. Anopl. Brit., 1842, p. 172, pl. XIV, fig. 3; Giebel, Insecta Epizoa, 1874, p. 216; Taschenberg, Die Mallophagen, 1882, p. 123; Kellogg, New Mallophaga II, 1896, p. 506, pl. LXVIII, figs. 4 and 6.

Four males and four females were collected from *Turtur chinensis* (Airyôshô, Distr. Nantô, May 4), from *Sphenocercus sororius* (Suisha, Distr. Nantô, May 4) and from *Graucalus rex-pineti* (Suisha, Distr. Nantô, May 3.).

Gen. COLPOCEPHALUM Nitzsch.

**13. *Colpocephalum pachygaster* Giebel.**

Giebel, Insecta Epizoa, 1874, p. 264; *Colpocephalum haliæti*, Denny, Monogr. Anopl. Brit., 1842, p. 216, pl. XIX, fig. 1.

Two males and six females of this species were obtained from *Pandion haliaetus* shot at Tamsui, Distr. Taihoku, May 22.

**14. *Colpocephalum osborni* Kellogg.**

Kellogg, New Mallophaga II, 1896, p. 521, pl. LXXI, figs. 2 and 3.

A male and a female were collected on *Milvus ater govinda* killed at Enteishô, Distr. Tainan, May, 9.

**15. *Colpocephalum umbrinum* var. *trilobatum* Giebel.**

*Colpocephalum trilobatum* Giebel, Insecta Epizoa, 1874, p. 275; Piaget, Les Pediculines, 1880, p. 557.

Two female specimens were collected on *Tringa subminuta* from Enteishô, Distr. Tainan, May 9.

Gen. *MENOPON* Nitzsch.

**16. *Menopon productum* Piaget.**

Piaget, Les Pediculines, 1880, p. 461, pl. xxxvii, fig. 8; Kellogg and Paine, Rec. Ind. Mus. X, 1914, p. 231.

A female specimen obtained from *Calophasis mikado*, shot on Mt. Arisan, May 11.

**17. *Menopon mikadokiji\** n. sp.**

(Plate X, fig. 7.)

A single male specimen was obtained from *Calophasis mikado* from Mt. Arisan, May 11.

*Description of male:*—Body length 1.27 mm., width 0.59 mm.; short, broad, pale brownish with distinct markings of blackish brown on head and indistinct transverse bands on abdomen.

Head length 0.29 mm., width 0.49 mm., widest through temples; front broadly parabolic, with indication of median angulation; several marginal hairs of different length and a few, very fine prickles on the frontal margin; a long hair on each lateral margin and two long hairs on the angle in front of each shallow ocular emargination; ocular fringe distinct, composed of stiff, curving hairs; palpi projecting by half the length of the last segment; temples round, expanded, bearing four long, pustulated hairs, several shorter hairs and a short spine; occipital margin concave, with six short hairs and

\* *Mikado* in Japanese means "Emperor," "kiji" "pheasant."

two fine spines. Colour of head pale brownish, with black, distinct ocular fleck and blackish brown blotches; occipital margin narrowly edged with brown, paler in the middle; mandibles and adjacent regions dark brown.

Prothorax length 0.15 mm. width 0.30 mm.; lateral angles blunt, each bearing a short spine; posterior lateral margins short, nearly straight, with two spines; the posterior margin convex, bearing three strong hairs on each side; transverse chitin bar pale but distinct, with the longitudinal bars at its ends. Metathorax length 0.2 mm., width 0.43 mm., with a faint sutural line setting off the mesothorax, which has nearly straight posterior margin; sides of metathorax straight, bare, diverging posteriorly; posterior angles bearing three spines; posterior margin truncate with a row of spines and hairs, interrupted at middle. Legs strong, slightly paler than body, with yellowish brown dorsal markings.

Abdomen oval, widest at the fourth segment, and each segment almost equal in length; posterior angles of segments projecting a little laterally, bearing one or two long hairs and two or three spines on segments I-VIII; the last segment broad, flatly rounded posteriorly, with two long hairs on each side and several short, weak hairs between them. Dorsal surface of each abdominal segment with a row of hairs of different length on each side of posterior margins. Ground colour of abdomen very pale yellowish brown, with a brownish transverse band, entirely across each segment. Genitalia distinct, of the usual *Menopon* type, longitudinal bar strong, reaching to the fifth abdominal segment and bearing two straight, sharp pointed rods.

### **18. *Menopon pallescens* Nitzsch.**

Giebel, Insecta Epizoa, 1874, p. 293; Piaget, Les Pediculines, 1880, p. 470, pl. XXXVIII, fig. 6; *Menopon perdicis*, Denny, Monogr. Anopl. Brit., 1842, p. 225, pl. XXI, fig. 9.

One female from *Arboricola crudigularis* taken on Mt. Arisan, May 14.

### **19. *Menopon longipectum* n. sp.**

(Plate X, fig. 2.)

One female specimen of this new species was obtained from *Arboricola crudigularis* shot on Mt. Arisan, May 14.

*Description of female* :—Body length 1.75 mm., width 0.66 mm. Ground colour of body very pale brownish, with distinct light pitchy markings on head; abdomen with narrow, brownish, marginal bands.

Head length 0.29 mm., width 0.45 mm., somewhat triangular in shape; front evenly rounded, with two short hairs at middle and on each side, in front of the ocular emargination, five marginal hairs and a few very fine prickles; a long and a short hair on the angle in front of each ocular emargination which is distinct; ocular fringe prominent, composed of numerous stiff hairs; palpi projecting by nearly the whole length of the last segment; temples expanded, margins somewhat angulated in front and behind, with four long pustulated hairs and a few short hairs and spines; occipital margin concave, straight at middle, with two short and two longer hairs. Colour of head very pale brownish; ocular fleck black, distinct; curved line bounding the antennal region pitchy inward, fading into dark brown outwardly; occipital margin edged with blackish brown, paler in the middle; mandibles pitchy; a brownish spot on the margin, just in front of each palpi.

Prothorax length 0.17 mm., width 0.29 mm., with slightly produced, blunt, lateral angles, each furnished with a short hair and two spines; posterior lateral margins weakly convex, bare; posterior margin flatly convex, with four hairs; transverse and longitudinal chitin bars pale, but distinct. Metathorax length 0.25 mm., width 0.45 mm.; with lateral emarginations and sutural line between meso- and metathoracic segments; lateral margins bare, diverging posteriorly; posterior lateral angles with two spines and a short hair; posterior margin weakly convex, with a long and three short marginal hairs on each side. Legs somewhat long and slender, concolourous with thorax, bearing a few short scattered hairs.

Abdomen broad, ovate; widening posteriorly to the fifth segment; then tapering more rapidly to the end; length of segments almost equal; posterior angles projecting, furnished on segments I-VII with two or three spines and a long hair; the latter a little apart from the angles; on segment VIII with two long and two short hairs; the last segment broad, rounded posteriorly with two long and a short hair on each side and a fringe of fine hairs on the posterior margin; dorsal surface of abdomen with a regular row of short hairs of different length on the posterior margin of each segment;

those on segments I-III complete and those on segments IV-VIII interrupted at middle. Colour of abdomen very pale brownish, with broad, smoky brownish lateral bands, which become indistinct on anterior segments and end at the eighth segment; brownish transverse bands present only on the ventral surface; those of segments I and II indistinct, those of segments VII to IX form one continuous blotch covering the whole space between the lateral bands, but the posterior half of the ninth segment is clear, with a narrow submarginal brownish band.

## 20. *Menopon urocissæ* n. sp.

(Plate X, fig. 5.)

Five females and a young were collected on *Urocissa caerulea* taken at Funbôshô, Distr. Nantô, April 29.

### Measurements.

	♀ mm.	♀ mm.	♀ mm.	♀ mm.	♀ mm.
Length of body.....	2.00	2.15	2.00	2.00	1.90
Width of body .....	0.84	0.84	0.86	0.88	0.88
Length of head.....	0.32	0.34	0.32	0.34	0.34
Width of head .....	0.64	0.65	0.65	0.67	0.65
Length of thorax .....	0.51	0.52	0.51	0.51	0.50
Width of thorax .....	0.62	0.62	0.62	0.64	0.63

*Description of female* :—Ground colour of body clear smoky brown, with brown and pitchy markings on the head; reddish brown lateral bands and brownish transverse bands on the abdomen.

Head somewhat lunate; front broad, slightly angulated, with eight marginal hairs and several fine prickles between projecting palpi; four long marginal hairs on the lateral margin, in front of each ocular emargination; a long and two short hairs on the dorsal surface on each side of the forehead; palp, projecting by half the length of the terminal segment; ocular fringe distinct, composed of numerous short hairs; temples narrow and expanded, each bearing six long pustulated hairs and several short hairs; occipital margin concavei with two short hairs at middle and two very fine spines on each side. Colour of head clear smoky brown, with black, prominent ocular fleck; curved line

bounding the antennal region pitchy inward, fading into brown outwardly; occipital margin edged with pitchy brown; two small triangular, brownish spots on the lateral margin of the front, outside of mandibles, which latter are pitchy brown, visible through the head.

Prothorax almost hexagonal in shape, with lateral angles much extended, bearing two spines and a short hair; posterior lateral margins slightly convex and bare; posterior margin convex, with three hairs on each side; transverse chitin bar clear, distinct; longitudinal bars brownish. Metathorax long, with slight lateral emarginations; indistinct sutural line separating mesothorax; lateral margins bare, strongly diverging posteriorly; posterior angles bearing three spines; posterior margin convex, furnished on each lateral part, a third of the whole in length, with a submarginal series of six or seven unequal hairs. Legs almost concolourous with thorax.

Abdomen broadly elliptical; widening posteriorly to the segment III, then tapering gradually to the end; lateral margins of segments almost equal in length, except three segments, one, two and nine, which are longer; posterior angles projecting, bearing three spines and a long hair on segments I-VI; three spines and two long hairs on segment VII and a spine and three long hairs on segment VIII; the last segment strongly rounded, with two long and two short hairs on each side and a fringe of fine hairs on the posterior margin. Posterior border of the first segment convex; of the second segment deeply concave; of third and fourth segments weakly concave and of the remaining segments almost truncate; posterior margin of the dorsal surface of each abdominal segment with a row of hairs of different length, interrupted at middle. Ground colour of abdomen paler than head and thorax; broad smoky brown lateral bands with the distinct sutures, ending at the eighth segment; no transverse bands on dorsal surface, but those of ventral surface showing through; ventral transverse bands of segments I-III indistinct; those of segments VIII and IX continuous, forming a blotch.

Gen. LÆMOBOTHRIUM Nitzsch.

**21. *Læmbobothrium loomisi* Kellogg & Chapman.**

Kellogg and Chapman, Journ. New York Ent. Soc. x, 1902, p. 23, pl. III, fig. 3.

A single female specimen of this species was obtained on *Nannocnus cinnamomea* from Enteishô, Distr. Tainan, May 9.

There can be no doubt about the present specimen being this species, for it agrees quite closely with the original description and figure, except for some points of slight difference about prothorax, although the specimens which they described were taken on quite a different host, *i.e.*, *Anser albifrons gambeli* (Sanfrancisco).

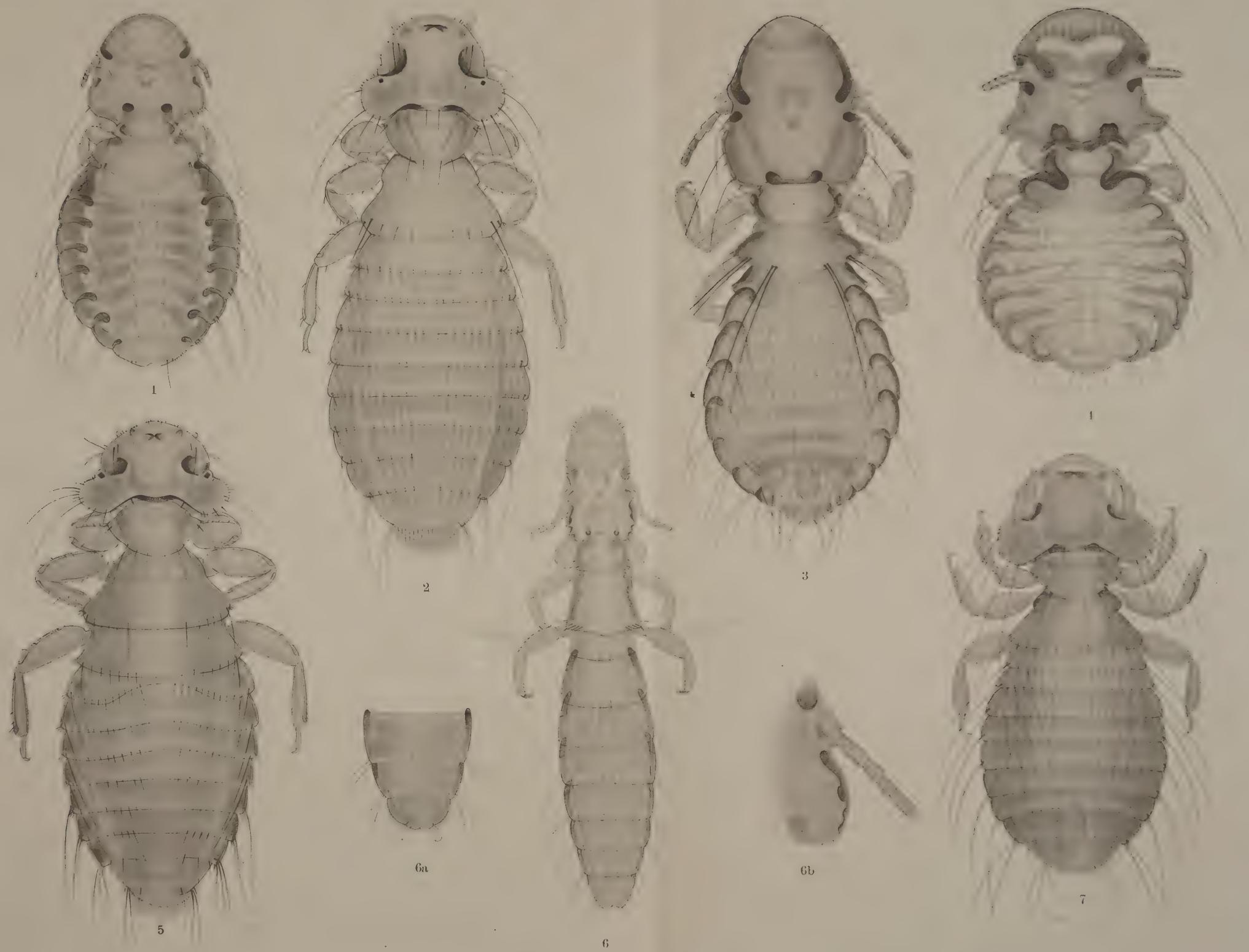
In the present specimen, the posterior margin between both posterior angles of prothorax is somewhat straight instead of being angular and anterior angles of prothorax bear four or five hairs instead of one; but these differences, of course, are not sufficient to separate it into subspecies.

Aug. 30, 1916.

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#### EXPLANATION OF PLATE X.

- Fig. 1. *Goniocotes microcephalus* n. sp., ♀. ×56.
  - Fig. 2. *Menopon longipectum* n. sp., ♀. ×65.
  - Fig. 3. *Nirmus ovatus* n. sp., ♂. ×70.
  - Fig. 4. *Goniocotes kurodai* n. sp., ♂. ×70.
  - Fig. 5. *Menopon urocissae* n. sp., ♀. ×50.
  - Fig. 6. *Lipeurus rubrifasciatus* Piaget, ♂. ×50.
  - Fig. 6a. *L. rubrifasciatus* Piaget; last abdominal segments of ♀. ×50.
  - Fig. 6b. *L. rubrifasciatus* Piaget; antenna and temple of ♀. ×120.
  - Fig. 7. *Menopon mikadokiji* n. sp., ♂. ×65.
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# Ueber das Askaron, einen toxischen Bestandteil der Helminten besonders der Askariden und seine biologische Wirkung.

(Mitteilung I.)

VON

Torai Shimamura und Hajime Fujii.

Mit 4 Textfiguren.

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### I. Einleitung.

Von den Störungen, welche die Helminthen in ihren Wirten hervorufen, sind die mechanischen Schädigungen und die Entziehung von Nährstoffen schon studiert und festgestellt. Neuerdings ist man weiter Vergiftungen durch toxische Produkte daran zu reihen gekommen, obgleich ihr Wesen noch nicht klar ist und einige Autoren die Anwesenheit von giftigen Substanzen in den Würmern in Zweifel ziehen oder gar verneinen wollen (BLANCHARD, SCHIMMELPFENNIG u. a.). Man kann aber aus zahlreichen Schriften über Helminthiasen klar erkennen, dass sich mehrere akute Fälle nur durch Vergiftung erklären lassen. Wenn die Symptome von Askariasisen bei Menschen und Tieren gewöhnlich nur Anämie, Abmagerung und nervöse Störungen sind, so ist doch auch tödlicher Ausgang mit Schwitzen, Kolik und Durchfall nicht selten (HAHMANN, DUNCAN, DANITZ, QUIRINO NERONI, GRÄFE, MERIEL, TRUELSEN RENDSBURG, GASTEIGER u. a.). Auch bei Punktur und Exstirpation von Echnokokkenblasen bei Menschen sind Ausbrüche von Urticaria und heftige Störungen berichtet, welche einen tödlichen Ausgang herbeiführten (CHAUFFARD, SCHLAGENHAUFEN, MOURON u. a.). Den Zoologen ist es ja bekannt, dass es empfindliche Individuen gibt, welche bei der Sektion und Behandlung von Askariden und Sclerostomen von schweren Augenreaktionen (Hyperämie und Schwellung von Konjunktiva und Kalaneula, Tränenfluss) und auch öfters von Urticaria auf der Stirn, Ohrenschwellung, Husten und Rhinopharyngitis befallen werden (BONDONY, BRAUN, ARTHUS et CHANSON, FAUST, HUBER, KANNEGIESSER, LEUKART, v. LINSTOW, PEIFER, GOLDSCHMIDT, WEINBERG u. a.). Im Jahre 1913 hat WEINBERG mit der Leibeshöhlenflüssigkeit von Askariden bei 256 Pferden die Augenreaktion durchgeführt und die folgenden Resultate erarbeitet:

1. Die Augenreaktion mit Askaridengift ist bei 168 unter 256 Pferden nur lokal positiv ausgefallen.
2. 10 Fälle wurden noch von Schwitzen und Dyspnoe begleitet und weitere 31 mit Diarrhöe.
3. Die Reaktion dauert nicht über 12–24 Stunden und die allgemeinen Symptome vermindern schon in 2–3 Stunden.

4. Die Reaktion fehlt bei Pferden, welche mit den Schmarotzern invasiert sind.

5. Sclerostomengift ruft auch Augenreaktion hervor, aber seine Wirkung ist etwas schwächer.

Seit MÜLLER und RIEDER in 1891 die Vermehrung von eosinophilen Zellen bei Ankylostomiasen bewiesen haben, ist von mehreren Forschern nicht nur die Veränderung der Blutbestandteile bei verschiedenen Helminthiasen bestätigt, sondern auch die Entstehung von Präzipitine und komplementablenkenden Antikörpern für Würmersubstanz bewiesen worden. In der letzteren Hinsicht sind die Untersuchungen über Echinokokkosen sehr zahlreich (WEINBERG, WELSCH und CHAPMAN u. a.) und die Komplementablenkung ist als so sicher durchführbar betrachtet, dass man das Verfahren zur Diagnostik von Echinokokkosen erfolgreich anwenden zu können behauptet.

Weiter bestätigen noch die Resultate von Tierversuchen mit Würmer-substanzen ihre Giftigkeit. CHANSON (1906) hat Kaninchen und Meerschweinchen die Leibeshöhlenflüssigkeit von Askariden injiziert und schwere nervöse Symptome und tödlichen Ausgang bei den letzteren beobachtet; DOBERNECKER (1912) hat auch bei subkutanen Behandlungen Paralyse und Exitus von Ratten, Meerschweinchen und Kaninchen konstatiert. F. FLURY (1912) hat mit der Leibeshöhlenflüssigkeit bei Hunden und Katzen bestätigt, dass einige unter Symptomen von Erbrechen, Kotdrang, Reflexerregbarkeit und Zuckungen zu Grunde gingen, und andere, welche lebend davon kamen, eine Resistenz gegen abermalige Injektion bekamen. Der Autor hat noch weitere chemische Untersuchungen über Askaridensubstanzen ausgeführt, und flüchtige Substanzen (Aldehyde, Fettsäuren, Estern, Alkohole), Prinbasen und Sepsinfektion als giftig bewiesen. Ausser den Askariden hat VAULGEARD (1911) mit *Taenia serrata* und ihrem *Cysticercus*, *Bothriocephalus punctatus* und *Moniezia neumanni* die gleichen Resultate konstatiert. Im Jahre 1914 hat SEYDERHELM Pferde mit dem Wasserextrakt von Gastrophiluslarven (*haemorrhoidalis et equi*) intravenös behandelt und schwere akute Symptome wie Würgebewegung, Schwitzen, Kotdrang, Hyperämie von sichtbaren Schleimhäuten und Ausbruch von Urticaria beobachtet. In schweren Fällen verendeten die Tiere in 4-5 Stunden nach der Injektion und bei der Autopsie wurden blutige Exsudate von Darmschleimhaut, endokardiale Blutung,

Hyperämie und Blutung in parenchymatösen Organen und unvollkommene Blutgerinnung beobachtet. M. OTA und H. DEGUCHI (1915) haben neben *Gastrophiluslarven* noch mit *Anoplocephala perfoliata*, *Sclerostomum vulgare et edentatum*, und *Filaria papillosa* die Ergebnisse von SEYDERHELM nachgeprüft.

Ueber die Toxizität der Echnokokkenflüssigkeit sind die Resultate von verschiedenen Seiten nicht eindeutig, VIRON, BOUDIN, und LAROCHE haben positive erhalten; die negativen Resultate von einigen Autoren sollen der zu geringen Dose von gebrauchter Flüssigkeit zuzuschreiben sein.

Aus dem oben Angeführten kann man allerdings richtig schliessen, dass die verschiedenen Helminthen gleiche giftige Produkte beherbergen, welche bei Versuchstieren identische Erscheinungen hervorrufen, aber es ist noch dunkel geblieben, was die Natur der toxischen Substanz oder Substanzen und was das Wesen der Vergiftung sei.

VAULLEGARD hat mit verschiedenen Helminthen gearbeitet und ein mit Alkohol fällbares „toxisches Ferment“ und „Alkaloide“ gewonnen, welche mit Alkohol aus dem Wasserextrakte nicht fällbar sind. Das „toxische Ferment“ wirkte auf die zentralen Nervenorgane und die letzteren auf die motorischen Nerven. SCHIMMELPFENNIG glückte es nicht aus Askaridenextrakte mittels STASS OTTO'scher Methode wirksame Alkaloide nachzuweisen. F. FLURY, welchem wir die Kenntnis über die verschiedenen giftigen Fraktionen von *Ascaris megalocephala* und *lumbricoides* verdanken, wollte die toxischen Substanzen der Würmer folgenderweise einteilen und ihrer Zusammenwirkung die Askaridenvergiftung zuschreiben :

1. Lokal reizende Substanzen . . . . . Flüchtige Gifte (Aldehyde, Fettsäuren, Alkohole und Ester).
2. Nervengift . . . . . Flüchtige Gifte und Purinbasen.
3. Capillargift . . . . . Sepsinfraktion.
4. Blutgift . . . . . Ungesättigte Fettsäuren (Oelsäure und Acrylsäure).

FLURY bemerkte, dass die Erscheinungen bei der Askaridenvergiftung und das Entstehen von der Resistenz denen bei serumaphylaktischen Schock sehr ähnlich sind; er ist jedoch zu keinem weiteren Schluss gekommen. SEYDERHELM meinte, dass nicht nur die Gastrophilusinjektion bei Pferden

„*Anaemia perniciosa infectiosa*“ herbeiführe, sondern dass auch das Blut von mit *Gastrophilus* behandelten Tieren die Krankheit auf den gesunden übertragen können. K. MUTO (1915) hat das Wesen von *Gastrophilus*-vergiftung bei Pferden studiert und seine Aufmerksamkeit besonders auf die Ähnlichkeit der Vergiftungssymptome mit denen des anaphylaktischen Shocks und auf die schnelle Entstehung von Resistenz gelenkt. Er hat mit dem Würmerextrakte als Substrat das ABDERHALDENSCHE Dialysierverfahren durchgeführt und mit den Seren aus nicht behandelten Pferden immer positive Reaktion und bei den aus behandelten negative erhalten. Daraus zog der Autor den Schluss, dass die *Gastrophilus*-vergiftung beim Pferde ein anaphylaktischer Vorgang sei, und dass die Ueberempfindlichkeit dem Tiere durch die Substanz von schon schmarotzenden Würmern verursacht werde.

GRÄTZ und DÉVÉ gehen so weit anzunehmen, dass der Shock bei der Echinokokkose durch in Echinokokkenblasen hineingelangtes, dort gespaltenes Eiweiss von Wirt verursacht werde.

Also ist man heute noch nicht zur Klarheit über das Wesen der Helminthenvergiftung gekommen, und die Natur der toxischen Substanzen ist noch dunkel. Man kann freilich sagen, dass in verschiedenen Helminthen identische Gifte beherbergt seien und die Vergiftungssymptome denen des anaphylaktischen Shocks ganz ähnlich. Unsere Frage wird demnach in die folgenden zwei geteilt:

1. Was sind die toxischen Bestandteile, welche unter den Helminthen so weit verbreitet sind?
2. Ist die Vergiftung mit der Ueberempfindlichkeit zu identifizieren?

## II. Chemisches über die toxischen Bestandteile.

### 1. VORVERSUCHE.

Da die Gewinnung von grossen Mengen verschiedener Helminthen nicht möglich ist, so haben wir als Hauptmaterial *Ascaris lumbricoides suis* gewählt, welche hier sicher gesammelt werden konnte und die Wirkung von andern Würmern mit der von den Askariden verglichen. Auch von frischen Askariden stand uns keine grosse Menge zur Verfügung. Wir haben täglich im Schlachthause die Würmer gesammelt und in Alkohol konserviert, bis die Menge gross genug war. Wir haben zuerst folgendes festgestellt:

1. Die Askaridensubstanz ist nicht nur bei Pferden sondern auch bei Meerschweinchen, Kaninchen und Hunden giftig wirksam. Unter den letzten drei ist das Meerschweinchen am empfindlichsten.

2. Der wässrige Extrakt des Askaridengiftes büsst seine Giftigkeit nicht ein, wenn es bei 100° C 6 Stunden erhitzt wird.

3. Der wässrige Extrakt von Askaridenpulver der in Alkohol aufbewahrten dann bei 50–60° C getrockneten Exemplare ist ebenso wirksam wie der Extrakt von den frisch getrockneten Würmern.

4. Die Leibeshöhlenflüssigkeit ist sehr stark giftig, unter den von Leibeshöhlenflüssigkeit ausgepressten verschiedenen Organen (Mundapparat, Geschlechtsorgane, Darmkanal und Fleischwand) konstatiert man aber keinen Unterschied von Virulenz.

5. *Ascaris lumbricoides* und *megalcephala* zeigen keinen Unterschied in ihrer Wirkung und Toxizität.

Die Resultate machen es wahrscheinlich, dass die giftigen Bestandteile von Askariden gegen verschiedene chemische Agenzien beständig seien, und dass es vorteilhaft sei, als Versuchsmaterial das Pulver von den in Alkohol konservierten Askariden und als Versuchstiere Meerschweinchen zu nehmen.

## 2. TOXIZITÄT DER VERSCHIEDENEN FRAKTIONEN DER WÜRMERSUBSTANZ.

Wir haben die in 90% igem Alkohol ca. 4–8 Wochen aufbewahrten Würmer bei 50–60° C schnell getrocknet und pulverisiert, dieses Rohpulver erst mit Aether dann mit 95% igem Alkohol so viel wie möglich von Fetten, Lipoiden und organischen Basen extrahiert, und zuerst die folgenden vier Materialien zum Versuche gehabt: 1) Aetherischer Extrakt, 2) alkoholischer Extrakt, 3) Rückstand aus dem Konservierungsalkohol und 4) mit Aether und Alkohol extrahiertes Rückstandpulver (das entfettete Pulver).

1). Der aetherische Extrakt beträgt 7,3% der Trockensubstanz vom Rohpulver und ist ein weißer Klumpen von Schweinefett ähnlicher Konsistenz mit buttersäurigem Geruch und der wässrige Extrakt davon bietet deutlich saure Reaktion. Wenn 0,5–1,0 g Substanz mit Sodalösung emulgiert und einem Meerschweinchen subkutan injiziert wird, zeigt das Tier keine Veränderung außer einem Juckkreisgefühl an der Impfstelle. Der wässrige Extrakt von 0,01–0,05 g Substanz ist intravenös auch ohne Wirkung. Der

Extrakt ist aber hämolytisch wirksam, und zwar kann 0,75 ccm 5% iger Extrakt 1,0 ccm 1% iges defibriniertes Pferdeblut vollkommen hämolsieren.

2). Der alkoholische Extrakt (die Ausbeute 4,2%) ist eine gelbbraune wachsartige Substanz mit dem Geruch von Leethin. Ein löslicher Teil in Aether wird mit Azeton gefällt. Der Extrakt löst sich in Wasser ziemlich gut und bietet saure Reaktion. Die Emulsion von 1,0 g Substanz kann nach Neutralisation mit Sodalösung einem Meerschweinchen ohne Wirkung subkutan injiziert werden; der wässrige Extrakt von 0,005–0,05 g Substanz intravenös einverlebt bewirkt im Tier auch keine Reaktion. 1 ccm 1% iges defibriniertes Pferdeblut wird von 0,5 ccm 1% igem Kochsalzextrakte vollkommen hämolytiert.

3). Aus dem Alkohol, welcher zu Konservierung von Würmern gebraucht wurde, gewinnt man einen ziemlich grossen Rückstand, dessen Beschaffenheit dem alkoholischen Extrakt gleich ist. Der Extrakt ist meistens mit Leibesflüssigkeit gemengt und kann toxisch sein. Die giftige Substanz kann zwar während des Aufbewahrens allmählich in die Flüssigkeit übergehen. Der alkoholische Extrakt vom Rückstand ist aber wirkungslos, 0,005–0,025 g Substanz intravenös nicht mehr toxisch. Er hat auch eine hämolytische Wirkung.

4). Das entfettete Pulver enthält 9,92% Stickstoff, 29,16 % Glykogen und 2,56% Asche. Nach dreimaliger Extraktion von Pulver, jedesmal mit einer 10 fachen Quantität Wasser für 2–3 Stunden, werden ca. 5% von Gesamtstickstoff und ca. 28% von Gesamtglykogen extrahiert. Der wässrige Extrakt ist milchig getrübt und von neutraler Reaktion; mit Essigsäure schwach angesäuert und gekocht lässt er kein Gerinnsel fallen. Die Flüssigkeit hat dieselbe Wirkung wie die Leibesflüssigkeit oder der wässrige Extrakt vom Röhpulver und ist hoch toxisch. Als letale Dosis für Meerschweinchen bei intravenöser Applikation ergibt sich aus dem Mittel von ca. 30 Fällen etwa 2,5 mg entfettetes Pulver, d. h., es ist 4 mal so stark wie das Röhpulver. Es hat keine hämolytische Wirkung für Pferde- und Ziegenblutkörperchen.

TABELLE I.\*

Material.	Nummer d. Meerschweinchen.	Gewicht g.	Applikation.	Dosis g.	Resultat.	Bemer- kung.
1. Aetherischer Extrakt.	272	190	wässriger Ext. (v)	0,01	negativ	
	273	160	„ „	0,05	„	
	270	205	Emulsion (c)	0,50	„	
	271	225	„ „	1,00	„	
2. Alkoholischer Extrakt.	95	360	wässriger Ext. (v)	0,005	„	
	101	355	„ „	„	„	
	285	130	„ „	0,025	„	
	286	150	„ „	0,050	„	
3. Rückstand v. Konservie- rungskalkohol. ditto mit abs. Alk. 2 mal extrahiert.	272	190	Emulsion (c)	1,000	„	
	114	240	wässriger Ext. (v)	0,0005	spur	
	94	320	„ „	0,0025	Exitus	
	274	185	„ „	0,005	negativ	
4. entfettetes Pulver.	—	—	„ „	0,0025	Exitus	Siehe Tab. IX. a.

F. FLURY hat die Aussicht geäussert, dass die toxischen Einflüsse von Askariden der Zusammenwirkung von mehreren giftigen Substanzen zugeschrieben werden müssen; er hat die Giftigkeit der sauren Destillate von den Würmern (Aldehyde, Ameisensäure, Propionsäure, Acrylsäure, Buttersäure, Valeriansäure, Aethylalkohol, Butylalkohol, Amylalkohol und deren Estern), Purinbase und Sepsinfaktion bestätigt und auch die Leibeshöhlenflüssigkeit als hoch toxisch konstatiert. Leider ist die Zahl der Versuche mit Tieren zu gering, als dass man daraus schliessen könnte, welches das Hauptgift sei. In unseren Versuchen hat der aetherische oder alkoholische Extrakt keine nennenswerten Störungen verursacht, daher müssen die flüchtigen Gifte und die in Alkohol löslichen Basen von FLURY entweder mit grosser Menge wirksam sein oder sein Gehalt ganz wenig. Unser entfettetes Pulver ist aber schon in kleiner Menge stark aktiv und hat dieselbe Wirkung, wie sie FLURY bei der Leibesflüssigkeitinjektion beschrieb. Obwohl die Purinbasen von FLURY schon in Dosen von 0,03–0,06 g bei Hunden und Katzen wirksam sind, ist aber 0,1 g Rohipulver (Puringehalt 0,45 mg) notwendig, um einen Hund zu töten.

\* (v) bedeutet intravenöse Injektion, (c) subkutane.

Daher muss das Hauptgift etwas anderes als flüchtige Substanzen, Purinen und in Alkohol lösliche Basen sein. Die hämolytische Wirkung von Askarien muss man mit FLURY dem aetherischen oder alkoholischen Extrakte zuschreiben, aber das Hauptgift wirkt keineswegs hämolytisch.

Der wässrige Extrakt von entfettetem Pulver wird nach Eindickung mit zwei Teilen von 95% igem Alkohol versetzt, um das Glykogen zu fällen. Aus dem klaren Filtrat wird weiter Syrup gemacht und dieser in absoluten Alkohol gegossen. Nun haben wir die folgenden drei Fraktionen: 1) Glykogenfraktion, 2) Askaronfraktion, 3) Filtrat.

1). Die Glykogenfraktion ist ein leicht im Wasser lösliches Pulver. Die Lösung gibt mit Jodlösung keine weinrote Färbung, aber mit Salzsäure gekocht reduziert sie erst FEHLINGSche Lösung und gibt mit Phenylhydrazin Osazone; mit Ammonsulfat halb gesättigt oder mit BRÜCKESchen Reagens und Salzsäure gibt sie nur wenige Niederschläge. 5,0 mg. Substanz intravenös einverleibt genügt nicht, um in einem Meerschweinchen Veränderungen hervorzurufen, 10 mg. davon wirkt zuweilen tödlich giftig.

2). Die Askaronfraktion ist stark toxisch. Als letale Dosis pro Meerschweinchen bei intravenöser Injektion ergibt sich aus ca. 50 Fällen 0,2–0,4 mg, d. h., dieses Gift ist zehnmal so wirksam wie das entfettete Pulver.

3). Das Filtrat enthält etwas Traubenzucker. 10 mg. Trockensubstanz ist atoxisch.

TABELLE II.

Nummer der Versuchstiere.	Gewicht g.	Material.	Dosis mg.	Resultat.	Bemerkung.
234	145	Glykogenfraktion	5,0	negativ	
236	125	"	10,0	Exitus	
233	120	Filtrat v. Askaronfraktion	5,0	negativ	
234	145	"	10,0	"	
—	—	Rohaskaron	0,2	Exitus	Siehe Tab. IX. b.

Es ist daher sicher, dass das Hauptgift der Askarien in der Askaronfraktion konzentriert ist, und man kann auch aus der Leibesflüssigkeit nach der Entfernung von gerinnbarem Eiweiss, mit derselben Operation eine Fraktion von identischer Wirkung und Toxizität bekommen.

Wenn man die Askaronfraktion mit Ammonsulfat weiter fraktioniert, und mit essigsaurem Baryum und kohlensaurem Ammon von Schwefelsäure und überschüssigem Baryum entfernt, gewinnt man noch die folgenden drei Fraktionen: 1) Primäre Albumosenfraktion, 2) sekundäre Albumosenfraktion, 3) Peptonenfraktion. Während zwischen den Wirkungen und der Toxizität von den drei Fraktionen kein Unterschied besteht, ist die Ausbeute von der zweiten Fraktion grösser und die von der dritten am wenigsten. Da wir aus den unten zu erwähnenden Evidenzen glauben, dass das Hauptgift der Askariden der Albumose-Peptonengruppe angehört, so haben wir für das Gift den Namen „Askaron“ und für die Askaronfraktion „Rohaskaron“ vorgeschlagen.

In der Tabelle III. wird die Vergleichung von Toxizität von verschiedenen Fraktionen aus Askariden für Meerschweinchen von 250–300 g. Körpergewicht zusammengefasst:

TABELLE III.

Material.	Dosis mg.	Resultat.
1. Trockensubstanz v. L. H. F.	0,5 (v)	Exitus
2. Rohpulver v. frischen Askariden	10,0 "	"
3. Rohpulver v. in Alk. konserv. Würmern	10,0 "	"
4. Entfettetes Pulver v. in Alk. k. Würmern	2,5 "	"
5. Rohaskaron aus Pulver	0,2 "	"
6. Rohaskaron aus L. H. F.	0,2 "	"
7. Primäre Albumosenfraktion	0,2 "	"
8. Sekundäre Albumosenfraktion	0,2 "	"
9. Peptonenfraktion	0,2 "	"
10. Aetherischer Extrakt	1000,0 (c)	negativ
11. Ditto. wässer. Extrakt	50,0 (v)	"
12. Alkoh. Extrakt	1000,0 (c)	"
13. Do. wässer. Extrakt.	50,0 (v)	"
14. Rückstand v. k. Alkohol	25,0 "	"
15. Glykogenfraktion	10,0 "	Exitus
16. Filtrat v. Rohaskaron	10,0 "	negativ

### 3. DARSTELLUNG VON ROHASKARON UND SEINE EIGENSCHAFTEN.

Das entfettete Pulver wird dreimal je mit einer zehnfacher Quantität Wasser 1–2 Stunden lang geschüttelt. Der vereinigte, milchig opaleszierende

Extrakt wird bei 50–60° C zu ca. 1/30 Volumen geengt und mit 2 Teilen 95% igem Alkohol versetzt. Das Gemisch wird eine Nacht stehen lassen, bis sich die Niederschläge von Glykogen und anderen Verunreinigungen von der überstehenden klaren grüngelben Flüssigkeit scharf abgeschieden haben. Das Filtrat wird weiter bei 40–50° C unter verminderter Drucke zum Syrupe eingedickt, und mit absolutem Alkohol versetzt, bis die Niederschläge nicht mehr neu entstehen. Nach 24 stündigem Stehenlassen saugt man die Niederschläge ab, dann werden sie erst mit absolutem Alkohol und mit Aether gewaschen und im Vakumexsikkator getrocknet. Die Ausbeute von Rohaskaron beträgt ca. 5% vom entfetteten Pulver.

Das Rohaskaron ist ein ziemlich hygroskopisches, weisses Pulver ohne Geruch und in Wasser und Alkohol (80%) leicht klar löslich. unlöslich ist es in Aether, Chloroform und Azeton. Es verbrennt mit dem Brenngeruch von Eiweisssubstanz und hinterlässt eine kleine Menge Asche. Die verdünnte Lösung (1%) ist wasserklar und von neutraler Reaktion. Eine Lösung von mehr als 0,1% iger Konzentration zeigt deutliche Farben- und Fällungsreaktion von Eiweiss, wie aus der Tabelle IV ersichtlich ist :

TABELLE IV.

Farbenreaktion und Fällung.	Rohaskaronlösung von		
	1%	0,1%	0,01%
Biuretreaktion	+	+	—
Ninhydrinreaktion	+	+	—
Xanthoproteinreaktion	+	+	—
MILLONSCHES Reaktion	Fällung +	±	—
	Farbung +	—	—
Tryptophanreaktion v. HOPKINS	+	±	—
Cystinreaktion	+	—	—
Dinzo-Reaktion	+	+	—
Histidinreaktion v. KNOOP	—	—	
Zusatz v. 3 fach. abs. Alkohol	+	—	
1/1 Sättigung v. Na Cl	—		
ditto (gekocht)	—		
ditto (mit Essig angesäuert)	+	±	—
1/2 Sättigung mit Na Cl (mit Essig angesäuert)	±	—	
ditto (Essig, gekocht)	+	—	

Farbenreaktion und Fällung.	Rohaskaronlösung von		
	1%	0,1%	0,01%
HELLERS Ringprobe	±	-	+
Ditto nach Sättigung m. Na Cl	+	±	-
Tannin	+	+	±
Pikrinsäure	+	+	±
BRÜCKES Reagenz	+	+	-
POLLACISCHE Reagenz	+	+	±
TSUCHYAS Reagenz	+	+	+

Das Rohaskaron reduziert die FEHLINGSche Lösung, während mit Ammoniumsulfat gefällte Präparate frei von reduzierenden Substanzen sind. Das Rohaskaron hat keine hämolytische Wirkung, 1 ccm 1%ige Rohaskaronlösung ist auf 1 ccm 1%iges defibriniertes Pferdeblut und 1 ccm 5%ige Ziegenblutkörperchen ohne Einfluss.

*Der Einfluss von Enteiweissung auf die Wirkung von Rohaskaron.* Das Rohaskaron enthält kein gerinnbares Eiweiß und es besteht hauptsächlich aus Substanzen von Albumose-Peptonencharakter. Kann man nun diese Albumose-Peptone selbst als das Hauptgift des Askaron betrachten? Um die Frage zu entscheiden haben wir folgenderweise den Einfluss von verschiedenen Enteiweisungen auf die Wirkung von Rohaskaron geprüft.

1). Ein Teil von Röhpulverwasserextrakt wird mit Essigsäure schwach angesäuert und bei 100°C 30 Minuten gekocht, und der andere nur gekocht. Nach dem Filtrieren mit einem Tonfilter büssen sie ein wenig ihre Toxizität ein, aber es ist kein Unterschied zwischen den beiden erkennbar. Erst eine Menge, die 5,0 mg entfettetem Pulver entspricht, ruft mittelstarke Reaktion hervor. Ein Teil von Askaron also wird von der Kerze adsorbiert.

2). Setzt man dem wässrigen Extrakt von entfettetem Pulver oder der Rohaskarolösung einen Zehntel ihres Gewichtes Tierkohle zu, so werden sie bei niedriger Konzentration nach einmaliger Filtration und bei höherer nach wiederholter Filtration frei von Eiweißreaktion und verlieren ihre toxische Wirkung, übrigens werden sie in 10 facher Quantität der letalen Dosis wirkungslos. Wenn das Filtrat von Tierkohlgemisch Eiweißreaktion zeigt, so ist es bei verhältnismäßig kleiner Menge immer toxisch. Die Wirkung geht somit seiner Eiweißreaktion parallel.

3). Wenn die Rohaskaronlösung mit Bleizucker und Bleiessig, Tannin, oder Kupferhydroxyd richtig enteiweisst wird und keine Eiweissreaktion bietet, so wird sie immer atoxisch. Wir haben einmal 1%ige Rohaskaronlösung mit Bleizucker und Bleisubazetat behandelt, bis keine Niederschläge mehr erschienen. Das klare Filtrat wurde mit Schwefelwasserstoff von überschüssigem Blei befreit und eingedickt. Obgleich die Flüssigkeit nur eine Spur von Biuretreaktion hatte, so reichte doch schon 0,5 ccm (10 fache Menge von Dosis letalis), ein Meerschweinchen zu töten. Die Flüssigkeit wurde dann weiter bis auf 2/3 eingedickt und mit Tierkohle einmal behandelt. Sie verlor nicht nur die Eiweissreaktion, sondern auch ihre Giftwirkung; 1 ccm davon (30 fache Menge von Dosis letalis) war atoxisch.

4). Ein 5%iger wässriger Extrakt von entfettetem Pulver wird in Schleichers Diffusionshülse, je 5 ccm, gebracht und gegen je 20 ccm Aussenwasser 40–60 Stunden dialysiert. Das Aussenwasser wird vereinigt und eingedickt. Wenn man die Hälfte des eingetragenen Giftes als Maximalbetrag der dialysierbaren Menge annimmt, wird die Flüssigkeit aus 50 mg entfettetem Pulver atoxisch und konnte diejenige aus 100–200 mg Pulver erst mittelstarke Reaktion verursachen.

Die Leibeshöhlenflüssigkeit, wenn sie in Diffusionshülsen gegen strömendes Wasser 70 Stunden dialysiert wird, büsst ihre Toxizität nur ein wenig ein.

Die oben erwähnten Resultate werden in der Tabelle V. resumiert.

TABELLE V. a.  
Versuchstier Meerschweinchen.

	Nr.	Gewicht g.	Material.	Dosis g.*	Resultat.
1.	43	230	1% wäss. Ext. v. entfett. Pulver mit Essig angescäuert, gekocht u. mit Tonkerze filt.	0,005	Exitus
	44	240	"	0,0025	stark
	49	250	5% wäss. Ext. v. entfett. Pulver mit Tonkerze filtrirt.	„	schwach
	50	270	"	0,005	mittel
	56	220	ditto, gekocht u. mit Tonkerze filt.	„	"
	57	210	"	0,01	"

\* g ist originaler Giftgehalt in der injizierten Flüssigkeit.

	Nr.	Gewicht g.	Material.	Dosis g.*	Resultat.
2.	52	270	ditto, mit Essig angesäuert, gekocht u. Tonkerze-behandlung.	0,005	negativ
	53	245	"	"	mittel
	54	225	"	"	schwach
	55	220	"	0,01	"
	36	266	1% wäss. Ext. v. entf. Pulver mit Tierkohle filtriert (Filtration 5 mal)	0,005	negativ
	37	355	"	0,01	"
	40	280	ditto 5% Ext. Tierkohlebehandlung 4 mal.	0,025	
	263	165	0,8% Rohaskaronlösung, Tierkohlebehandlung 3 mal.	0,001	
	264	"	"	0,002	"
	42	232	1% wäss. Ext. v. entf. Pulver, mit Tannin enteiweisst.	0,125	"
3.	51	220	ditto, mit Bleizucker u. Bleiessig behandelt.	"	"
	62	320	5% wäss. Ext. mit Kupferhydroxyd behandelt.	"	"
	45	320	40 stund. Dialysat v. 5% wäss. Ext.	0,0025	"
	46	290	"	0,06	schwach
	47	320	"	0,12	mittel
	59	185	60 stund. Dialysat.	0,04	negativ
	60	195	"	0,25	mittel
4.	61	185	"	0,125	"
	177	160	gegen strömend. Wäss. 70 Stunde dialysierte Leibeshöhlenflüssigkeit.	0,1ccm	Exitus

TABELLE V. b.

Versuchstier Pferd.

Namen.	Material.	Dosis g.*	Result.
Akiyama	2% wäss. Ext. v. Rohpulver mit Tierkohle 6 mal behandelt.	0,5	schwach
Mikadzuki	ditto, mit Bleizuker u. Bleisubazetat behandelt.	"	negativ
Hakusan	"	"	"
Rikuni	84 stund. Dialysat v. 5% wäss. Ext. v. Rohpulver.	"	"

Aus obigen Resultaten ist es klar, dass das Askaron ein nur langsam diffusibles und von verschiedenen Suspensoiden adsorbierbares Kolloid ist. Es geht nicht in enteiweisstes Filtrat über, und seine Giftigkeit geht mit der

\* g ist originaler Giftgehalt in dernj iizierten Flüssigkeit.

Eiweissreaktion seiner Lösung parallel. Daneben findet sich ein so grosser Unterschied von Toxizität des Rohaskarons nach seinen verschiedenen Applikationsarten, dass die letale Dosis für Meerschweinchen bei der subkutanen Injektion 100 mal so gross sein muss wie bei der intravenösen (Siehe Tierversuche). Die genannte Tatsache zwingt uns das Askaron als eine Kolloidesubstanz und mit der Albumose-Pepton von Rohaskaron als identisch zu betrachten.

FLURY bemerkte bei der Analyse der Askaridensubstanz die Anwesenheit von höheren Eiweisspaltprodukten, jedoch schreibt er nichts über ihre Wirkung. WEINBERG behauptet ohne Beweis die Ungiftigkeit von Albumose-Pepton in der Leibeshöhlenflüssigkeit von Askariden.

*Resistenz von Askaron.* Das Askaron ist gegen Erhitzung, Säure, Alkali, künstliche Verdauung und Sonnenbeleuchtung sehr stabil, und seine Toxizität vermindert sich über ein Jahr lang nicht, wenn das Rohaskaron im Exsikkator aufbewahrt wird.

1. Erhitzung. Die Rohaskaronlösung wird bei 100, 130 und 150° C je eine Stunde erhitzt. Im ersten Falle büsst sie ihre Giftigkeit nicht ein, bei 130° C ein wenig, und bei 150° C zwei Drittel davon.

2. Säure und Alkali. Die Rohaskaronlösung (3,2%) wird mit gleichem Volumen N/5-N/1 Salzsäure bzw. N/5 Natronlauge versetzt und 20-30 Minuten bei 100° C gekocht. Die verdünntere Salzsäure hat keinen Einfluss auf ihre Wirkung, bei den anderen Fällen verliert sie aber die Hälfte ihrer Toxizität.

3. Sonnenbeleuchtung. Das entfettete Pulver wurde in einem Reagenzglas eingeschlossen und während des Sommers ca. 50 Tage lang der Sonnenbeleuchtung unterworfen. Es verlor die Hälfte seiner Toxizität.

4. Künstliche Verdauung. Das Rohaskaron wird mit einer ca. 3 facher Menge Trypsin (Grübler) bzw. Pepsin (Riedela) 14-60 Stunden verdaut. Bei der Pepsinverdauung bleibt die Toxizität ohne Veränderung, bei der ersten aber wird eine kleine Verminderung konstatiert.

TABELLE VI.  
Beständigkeit von Askaron.

	Nr. v. Meersch.	Gewicht g.	Material.	Dosis mg.	Resultat.	Bemerkung.
1.	12	265	0,5% wäss. Ext. v. entfett. Pulver bei 100° C 1 Stunde gekocht.	1,0	mittel	
	11	290	"	2,5	Exitus	
	15	280	"	"	"	
	33	275	ditto 1% wäss. Ext. 130° C, 1 Stunde.	5,0	stark	
	34	280	"	7,0	mittel	
	35	325	"	10,0	sehr stark	
	261	170	0,8% Rohaskaron, 130° C, 1 Stunde.	0,25	sehr schwach	Kontrolle
	253	195	"	0,8	Exitus	L. D. = 0,5 mg
	31	295	1% wäss. Ext. v. entf. Pulver, 150°C, 1 Stunde,	5,0	schwach	
	32	270	"	10,0	Exitus	
	267	170	0,8% Rohaskaron, 150° C, 1 Stunde.	0,8	stark	Kontrolle
	265	160	"	1,6	Exitus	L. D. = 0,5 mg
2.	28	210	2% wäss. Ext. v. entf. Plv. + N/5 HCl, 100° C, 20 Minuten gekocht.	5,0	"	
	29	270	"	2,5	"	
	257	160	3,2% Rohaskaron + N/1 HCl, 100° C, 30'.	0,5	mittel	Kontrolle
	258	160	"	1,0	"	L. D. = 0,5 mg
	30	320	2% wäss. Ext. v. entf. Plv. + N/5 NaOH, 100° C, 20'.	2,5	negativ	
	26	245	"	5,0	schwach	
	27	297	"	10,0	Exitus	
	262	160	3,2% Rohaskaron + N/5 Na OH, 100° C, 20'.	0,4	negativ	Kontrolle
	260	175	"	0,8	Exitus	L. D. = 0,4 mg
3.	92	315	50 tägige Sonnenbleuchtung v. entfett. Pulver.	2,5	negativ	
	100	295	"	5,0	mittel	
4.	132	220	Rohaskaron mit Pepsin verdaut, 14 Stunde.	0,3	Exitus	Kontrolle
	133	212	ditto, Trypsin, 14 Stunden.	..	"	L. D. = 0,2 mg
	134	274	"	0,2	"	
	290	130	ditto, Pepsin, 60 Stunden.	0,4	"	
	292	190	"	"	"	
	289	125	ditto, Trypsin, 60 Stunden.	0,8	"	Kontrolle
	291	195	"	"	stark	L. D. = 0,4 mg
	293	210	"	"	Exitus	

### III. Tierversuche.

#### I. SYMPTOMATOLOGIE.

##### a. Pferde.

###### 1. Allgemeine Symptome.

Das Askaron ruft bei Pferden intravenös oder subkutan einverleibt allgemeine Symptome hervor. Die Hauptsymptome sind Hyperämie der sichtbaren Schleimhäute, Vermehrung der Absonderungen (das Schwitzen am deutlichsten, Nasenschleimfluss und Speichelabsonderung auch deutlich), Kotdrang, Atemnot, Oedem (an den Konjunktiven, Nasenflügel und Lippen deutlich), Tachykardie, Zuckung, Krämpfe, Kolikanfälle, Depression und auch Ausbruch von Urticaria (selten).

Der Verlauf ist meistens schnell, nach 30 Minuten erreicht er das Maximum und nach 2–3 Stunden verschwinden alle Symptome; das Tier kann aber manchmal mit Enteritis 1–2 Tage lang kranken, und endlich verenden. Bei Anwendung von grösserer Menge Gift geht das Versuchstier regelmässig noch mit Symptomen von Kolikanfällen und Herzschwäche in 4–5 Stunden zu Grunde, auch kann es mit schwerer Dyspnoe und heftigen Krämpfen schon nach 10 Minuten verenden. Die Sektionsbefunde zeigen hämorrhagische Infiltration der Darmschleimhaut und endkardiale Blutung, Ekchymosen und Trübung der parenchymatösen Organe.

Hier sind einige Beispiele aus dem Protokolle gegeben.

Das Rohaskaron wird in 10–20 ccm physiologischer Kochsalzlösung gelöst und sofort zur Injektion benutzt. Das Rohpulver wird mit 15–30 ccm 0,85% iger Na Cl Lösung ca. eine Stunde in Mörser zerreibend extrahiert und mit Papier filtriert.

#### FALL I.

Den 28. Feb. 1916.

Pferd Kotogawa 14 Jahre alt, Körpergewicht von 350 kg, Ernährungszustand gut.

Stunde.	P.	A.	T.	Symptome.
10° 35'	36	12	37,0	Normal.
40	—	—	—	1,0 mg Rohaskaron intravenös.

Stunde.	P.	A.	T.	Symptome.
41	—	—	—	Kotabsatz, Tränenfluss, Zuckung, Hyperämie der Konjunktiven.
43-45	—	—	—	Kotentleerung, schaumiger Speichel aus dem Maule, schwitzt anfangs an Hals, Leistengegend, Hodensack und dann auf dem ganzen Körper, Kotdrang, der Schweif immer aufgehoben.
46-50	—	—	—	Kotabsatz, Nasenschleimfluss plasmaartig, Drängen, Dyspnoe, Kolikanfälle, Schweiß fliesst tropfenweise herab.
50-55	42	24	36,9	Schwitzen, Nasenschleimfluss, Salivation.
56	—	—	—	Hyperämie der sichtbaren Schleimhaut verschwindet allmählich, Kolik.
11° 0'	42	48	—	Dyspnoe.
2	—	—	—	Kotentleerung, Kot mit gelbem Schleim überzogen.
3	—	—	—	" "
5	—	—	—	„ Konsistenz weich.
8	—	—	—	" "
10	—	—	—	" "
13	—	—	—	" "
15	—	—	—	Diarrhöe mit Blut, Kolik, Depression.
20	—	30	—	Drängen.
21	60	—	36,1	Drängen, Herztöne schwach, Puls nicht fühlbar.
23	—	—	—	Diarrhöe.
24	—	—	—	"
25	—	—	—	"
28	—	—	—	"
30	—	—	—	Kotdrang.
32	—	—	—	"
34	—	—	—	"
36	—	—	—	„ Schwitzen und Nasenschleim- u. Tränenfluss hören auf.
39	—	—	—	Blutige Diarrhöe.
45	60	36	36,0	Diarrhöe, Erschlaffung des Sphinkter ani, Kolik.
46	—	—	—	Blutige Diarrhöe, Depression, Entkräftigung.
47-50	—	—	—	Augenlider geschlossen, Zunge hängt herab, Drängen.
51	—	—	—	Diarrhöe.
54	—	—	—	"
58	—	—	—	"
12° 0'	72	42	—	"
4	—	—	—	"
10	—	—	—	"

Stunde.	P.	A.	T.	Symptome.
15	—	—	—	Drängen, Konjunktiva werden wieder injiziert.
16	—	—	—	Blutige Diarrhöe.
30	90	30	36,5	Heftige Kolik.
31	—	—	—	Blutige Diarrhöe.
36	—	—	—	Kolik noch dauernd.
45	—	—	—	Zungenparalyse.
1° 0'	—	—	—	Blutige Diarrhöe.
10	—	—	—	Atmung tief, Kolik.
20	78	18	36,7	
22	—	—	—	Hauttemperatur sinkt allmählich.
30	—	—	—	Dyspnoe. Es schwankt.
57	—	—	—	Es fällt auf den Boden.
2° 13'	—	—	—	Es steht wieder auf.
16	84	—	—	Es stürzt: Seitenlage genommen; Herzschläge schwach.
28	—	—	—	Erweiterung v. Pupillen, Kolik.
31	—	—	—	Zuckung und Krampf.
37	—	—	—	Harnlassen, Atemstillstand, Exitus.

Sektionsbefunde: Diffuse Blutung von Dünndarm, Stauung und Echymose von Magen, Dickdarm und Trachea. Endokardiale Echymose, Lungen- und Nierenhyperämie, Blut unvollkommen geronnen und teerartig. Gastrophiluslarven im Magen gefunden.

#### FALL 2.

Den 21. März 1916.

Kirifu, Fuchs, 16 Jahre alt, 345 kg Körpergewicht. Ernährungszustand etwas schlecht. Appetit gut.

Das Pferd wurde zum Zwecke der Immunisierung 17 mal mit steigenden Giftmengen behandelt, und es hat bei der letzten 0,3 g Rohaskaron überstanden.

Stunde.	P.	A.	T.	Symptome.
10° 55'	42	26	37,1	Normal.
57	—	—	—	0,5 g Rahaskaron intravenös.

Stunde.	P.	A.	T.	Symptome.
11° 9'	-	-	-	Sofort nach der Injektion erregt, schwankt mit der Parese der Hinterbeine, stürzt nieder, Dyspnoe, tiefe und lange Atmung; Konjunktiva, Nasen- und Mundschleimhaut hoch injiziert.
10	-	-	-	Zuckung, Krämpfe, leichtes Schwitzen, Atemstillstand, Krampf, Kot- und Harnentleerung.
11	-	-	-	Exstus.

Autoptische Befunde: Lungenblutung und Stauung, Nierenblutung, Blutergerinnung unvollkommen, Psammom cerebri, Scharikosis hepatis.

## 2. Die letale Dosis für Pferde.

Obgleich die individuelle Empfindlichkeit bei Pferden ziemlich divergent ist und unsere Versuchsfälle allerdings zu wenig, so können wir doch mit Sicherheit die letale Dosis von Rohaskaron bei intravenöser Anwendung als 1,0 mg pro Kopf, d. i. 0,004 mg pro kg Lebendegewicht schätzen.

TABELLE VII.\*

Datum.	Namen der Pferde.	Material.	Anwendung.	Dosis mg.	Resultat.
4/III/15	Mikazuki	Rohpulver (A. m)	(v)	100,0	negativ
11/III	Kaga	Rohpulver v. inneren Organen(A.m)	(c)	1000,0	Exstus
24/III	Rikugun	Rohpulver v. Darmkanal (A. m)	(v)	200,0	"
29/III	Ohmi	"	(c)	50,0	negativ
13/VIII	Akiyama	Rohaskaron II. (A. l.)	(v)	1,0	Exstus
29/IX	Yuwane	Rohaskaron IV. (A. l.)	(c)	0,2	sehr stark
12/X	Shuntei	"	"	0,1	mittel
23/XI	Kirifu	"	"	0,2	negativ
24/XI	Yamasakura	"	(v)	0,5	schwach
2/I/16	Shiki	"	"	0,8	"
16/I	Shichitei	"	(c)	0,1	"
21/II	Asabu	"	(v)	0,1	mittel
28/II	Kotogawa	"	"	1,0	Exstus
13/III	Shinagawa	"	"	0,5	stark
8/IV	Shumpo	"	"	1,0	Exstus
6/V	Hokuyaku	"	"	1,0	"

\* Die römische Figur bedeutet die Darstellungsnummer von Präparaten. A. l., *Ascaris lumbricoides*; A. m, *Asc. megalcephala*; v, intravenös, c, subkutan.

## 3. Lokale Reaktion.

Subkutane und intrakutane Injektion von Rohaskaron rufen bei Pferden keine lokale Reaktion hervor; aber Einräufelung von nur 0,01% iger Rohaskaronlösung schwere Augenreaktion. Die Symptome sind heftige Bewegung der Membrana nictitans, Hyperämie der Konjunktiva, Tränenfluss, Oedem der Konjunktiven und Augenlider: unsere Resultate stimmen mit den Ergebnissen von WEINBERG ganz überein. Der Verlauf ist hier auch schnell und die Symptome verschwinden in 1-2 Stunden, obgleich schwerer Oedem 12-24 Stunden lang dauern kann. Bei wiederholter Behandlung wird die Reaktion allmählich schwächer, verschwindet aber nicht.

TABELLE VIII.

## Augenreaktion.

Datum.	Name der Versuchspferde.	Material.	Resultate.	Bemerkung.
22/IX/'15	Yuwane	4:10000 Rohaskaron IV.	mittel	
"	Ohmi	"	negativ	Eine Stunde vor d. Einräufelung von 0,4 mg Rohaskaron IV subkutan behandelt.
"	Terashima	"	stark	
23/IX	Retsugo	"	negativ	
12/X	Shuntei	1:10000	mittel	
9/XI	Karutobi	"	schwach	links
"	"	2:10000	"	rechts
"	Yokonami	"	stark	
"	Koen	1:10000	schwach	
"	Chitose	"	mittel	links
"	"	2:10000	stark	rechts
"	Shomei	1:10000	mittel	
"	Seiman	"	schwach	
"	Nanano	"	"	
12/XI	Kiicho	2:10000	mittel	
"	Nancho	1:10000	schwach	
"	Shumpo	2:10000	mittel	
"	Kendai	1:10000	"	
"	Kirifu	"	stark	

Datum.	Name der Versuchspferde.	Material.	Resultate.	Bemerkung.
12/XI	Torin	1:10000	stark	
"	Yamasakura	2:10000	"	
"	Nishiki	"	"	
"	Shunketsu	"	"	
"	Genya	"	mittel	
"	Asabu	"	schwach	
"	Shichitei	"	"	
17/XI	Nishin	1:1000	stark	
3/V/'16	Hikoki	1:1000 Rohaskaron aus Gastrophiluslarven.	sehwach	links
"	"	"	"	rechts
"	Hokuyaku	1:100 Rohpulver v. Sclerostomen.	"	links
"	"	"	"	rechts

## b. Meerschweinchen.

## 1. Intravenöse Injektion.

Die Hauptsymptome von Askaronvergiftung bei Meerschweinchen sind Erregung, Kaubewegungen, krampfhaftes Husten, Dyspnœ, Atemstillstand und Anfälle von Krämpfen, und das Tier verendet in ca. 10 Minuten. In nicht tödlichen Fällen folgen noch Zittern, Entkräftigung, Depression, Temperaturstürze (Schwankung von ca. 5°C nicht selten) und Herzschwäche, welche regelmässig in 1-4 Stunden verschwinden. Die Sektionsbefunde sind hochgradige Lungenblähung, epikardiale Ekchymosen und unvollkommene Blutgerinnung.

## BEISPIEL 1.

Den 28. Sept. 1915. Meerschweinchen Nr. 129, Körpergewicht 210 g.

Stunde.	Temp.	Symptome.
1° 35'	38,3	Normal.
36	—	0,2 mg Rohaskaron intravenös.
37	—	Würgebewegung, Husten, Dyspnœ, krampfhaftes Atmen, Krampfanfälle.
38	—	Seitenlage, Krämpfe, Atemstillstand.
40	—	Agonale Atmung.
42	—	Herzschlag nicht fühlbar, Exitus.

Sektionsbefunde. Lungenblähung, Blutgerinnung unvollkommen.

## BEISPIEL 2.

Den 18. Sept. 1915. Meerschweinchen Nr. 125, Körpergewicht 235 g.

Stunde.	Temp.	Symptome.
4° 25'	39,0	Normal.
27		0,2 mg Rohaskaron intravenös.
28-41		Dyspnoe, Würgebewegung, unruhiges Benehmen, Anfall von krampfhaftem Husten, Atmung krampfhaft, Zittern, Kratzen am Gesichte.
42	38,0	Atemfrequenz grösser, zur normalen.
57	37,5	Symptome verschwinden.
5° 12'	36,9	Depression, die Haare sträuben sich.
27	37,0	Entkräftigung, Zittern.
42	37,1	
57	37,2	
6° 12'	37,9	Zittern noch dauernd.
42	37,7	"
7° 22'	38,3	Etwas munterer.
52	38,1	
8° 22'	38,2	
9° 22'	38,5	
52	38,6	
10° 22'	39,0	Erholt.

Um die Temperaturstürze ersichtlich zu machen, lassen wir die typischen Kurven (Fig. 1-2) folgen.

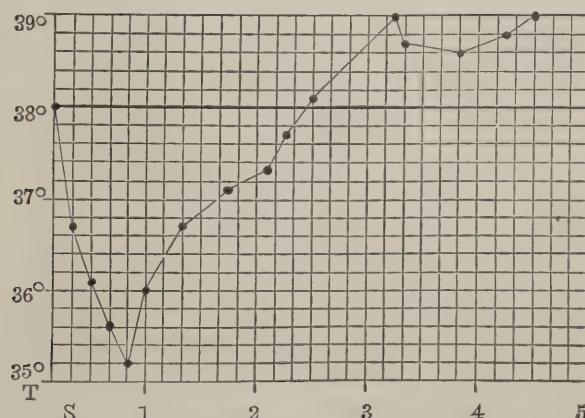


Fig. 1. Temperaturkurve. Nr. 1. 0,001 g Rohpulver intravenös.

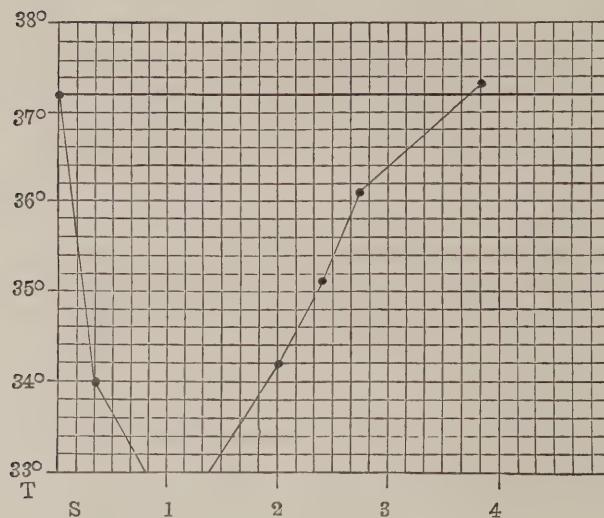


Fig. 2. Temperaturkurve. Nr. 248. 0,4 mg Rohaskaron  
intravenös.

## 2. Die letale Dosis.

Bei Meerschweinchen beträgt sie 2,5 mg von entfettetem Pulver bzw. 0,2–0,4 mg Rohaskaron, wie man aus den Tabellen IX a–b einsehen kann.

TABELLE IX. a.

Intravenöse Injektion von Wasserextrakten von entfettetem Pulver.

Datum,	Nummer,	Körpergewicht g.	Dosis g.	Resultat.
21/IV/15	1	300	0,001	stark
"	2	355	0,002	"
"	3	"	"	sehr stark
22/IV	4	255	0,003	Exitus
"	5	285	0,002	"
23/IV	6	260	0,0005	schwach
"	7	"	0,001	sehr stark
26/IV	8	210	0,003	Exitus
"	9	220	"	negativ
"	10	190	0,001	schwach
"	11	290	0,0025	Exitus
"	12	265	0,001	stark

Datum.	Nummer.	Körpergewicht g.	Dosis g.	Resultat.
26/IV	13	265	0,0005	schwach
"	14	295	0,001	"
"	15	280	0,0025	Exitus
28/VI	16	"	0,001	"
"	17	300	0,0005	mittel
"	18	240	"	schwach
"	19	260	"	mittel
"	20	215	"	"

TABELLE IX. b.\*

## Intravenöse Injektion von Rohaskaron.

Datum.	Nummer.	Körpergewicht g.	Material.	Dosis mg.	Resultat.
12/VIII/15	77	290	Rohaskaron I.	0,04	schwach
"	78	295	"	0,5	Exitus
"	79	264	"	0,1	"
14/VIII	81	240	Rohaskaron II.	0,066	schwach
"	82	218	"	0,13	Exitus
8/IX	111	305	Rohaskaron III.	0,1	negativ
"	112	335	"	0,4	schwach
10/IX	121	350	"	1,0	"
17/IX	122	325	Rohaskaron aus Leibeshöhlenflüssigkeit.	0,2	"
"	123	240	"	"	Exitus
"	124	240	"	0,1	mittel
18/IX	34	440	sekundäre Albumose aus Rohaskaron III.	1,0	Exitus
"	93	400	"	0,5	stark
"	125	235	"	0,2	"
28/IX	128	230	Rohaskaron IV.	0,1	"
"	129	210	"	0,2	Exitus
1/X	131	315	"	0,5	"
16/X	154	180	primäre Albumose aus Rohaskaron IV.	0,2	"
"	155	210	"	0,08	"

\* Römische Figur nach "Rohaskaron" zeigt die Nummer der Präparaten.

Datum.	Nummer.	Körpergewicht g.	Material.	Dosis mg.	Resultat.
16/X	156	205	sekundäre Albumose aus Rohaskaron IV.	0,2	Exitus
"	157	172	"	0,1	sehr stark
"	158	167	Pepton aus Rohaskaron IV.	1,0	Exitus
"	159	237	"	0,4	"
"	160	247	"	0,08	negativ
"	161	252	"	0,2	Exitus
20/X	162	130	"	0,1	schwach
"	163	230	"	0,2	Exitus
1/XI	169	435	Rohaskaron IV.	0,1	negativ
3/XI	170	355	"	0,2	schwach
22/11/16	187	280	"	"	Exitus
"	188	360	"	"	schwach
"	190	260	"	"	Exitus

### 3. Intraperitoneale Injektion.

Die Erscheinungen bei intraperitonealer Injektion, welche weit langsamer als bei der intravenösen verlaufen, sind hauptsächlich Kaubewegung, Dyspnoe, Parese der Hinterbeine, Entkräftigung, Zittern, Temperatursenkung, Herzschwäche; der Verlauf dauert über 6-12 Stunden. Bei autoptischer Sektion konstatiert man schwache Lungenstarre, Blutung der Magendarmschleimhaut und der serosen Höhlen und Endkard. Die Blutgerinnung ist auch unvollkommen.

#### BEISPIEL 1.

Nr. 212. Gewicht 135 g.

Stunde.	Temperatur.	Symptome.
11° 0'	39,5	Normal.
4	-	0,05 g. Rohaskaron intraperitoneal.
5-10	-	Dyspnoe, Depression, die Haare sträuben sich.
12	-	Krampfartige Atmung, Kratzen an der Nasenspitze.
14	-	"
19	34,5	
27	-	Atmung wird etwas ruhiger, Depression.
30	-	Parese der Hinterbeine, Atemnot.
37	unter 34° C	

Stunde.	Temperatur.	Symptome.
55	unter 34° C	
12° 15'	"	Entkräftigung, Herzschwäche.
45	"	
1° 15'	"	Zittern, Depression, Parese der Hinterbeine.
50	"	
2° 20'	"	
3° 30'	"	
5° 0'	"	Heftiges Zittern, motorische Parese.
30	"	Cyanose v. Konjunktiven und Sohlen, Ohren kalt, Herzschlag schwach und klein.
6° 10'	"	Es legt sich nieder, Atem beschleunigt und klein.
7° 5'	"	Kollaps. Exitus.

Sektionsbefunde. Lungenhyperämie und Hydrops, endokardiale Ekchymose, Blutgerinnung unvollkommen.

#### BEISPIEL 2.

Nr. 21. Gewicht 250 g.

Stunde.	Temperatur.	Symptome.
1° 54'	39,0	Normal.
2° 0'	—	0,25 g entfettetes Pulver intraperitoneal.
5-9		Kaubewegung, Dyspnoe, unruhig, Reflexerregung.
10		Dyspnoe, es legt sich.
15	36,7	
20	—	Kann nicht aufstehen. Kolik (?). Depression.
30	36,1	
40	—	Lähmung, Parese der Hinterbeine.
45	unter 35,0	
50	—	Cyanose der Konjunktiven, Bauchgegend und Sohlen: Herzschlag schwach, Kratzen am Gesichte.
3° 10'	—	Es schwankt, Atemfrequenz vermehrt.
15	—	Krampf, Zuckung der Hinterbeine, Opisthtonus.
20	—	Anfall v. Zuckungen, Ohren und Extremitäten kalt.
35	"	
4° 20'	"	
25	—	Noch mit Fresslust.

Stunde.	Temperatur.	Symptome.
35	—	
50		Es schreitet ein wenig.
5° 30'	35,6	Allgemeine Symptome bessern sich.
6° 0'	36,1	
30	37,3	Noch zitternd.
8° 30'	38,1	Symptome verschwinden.
9° 30'	39,1	Erholt.

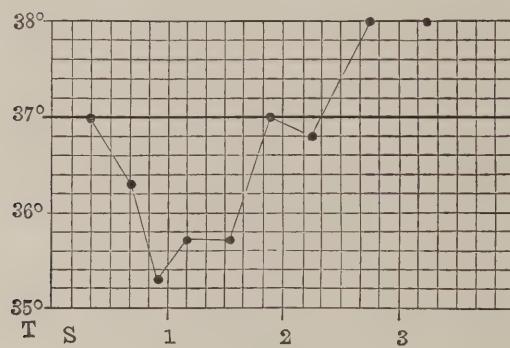


Fig. 3. Temperaturkurve.  
Nr. 216. 25 mg = 2 ccm Rohaskaron intraperitoneal.

Die letale Dosis bei intraperitonealer Injektion beträgt ungefähr 50,0 mg Rohaskaron (200,0 mg pro kg Körpergewicht).

#### TABELLE X.

##### Intraperitoneale Injektion.

Datum.	Nummer.	Gewicht g.	Material.	Dosis g.	Resultate.
29/IV/15	21	250	Entfettetes Pulver.	0,25	sehr stark
"	22	270	"	0,125	schwach
"	23	280	"	0,5	mittel
25/III/'16	216	138	Rohaskaron IV.	0,025	"
"	212	132	"	0,05	Exitus
"	197	310	"	0,1	"

#### 4. Subkutane Injektion.

Die Symptome und Sektionsbefunde bei der subkutanen Injektion sind gleich wie die bei intraperitonealer, der Verlauf der Reaktion ist aber noch langsamer. Die die Reaktion herbeiführende Dosis ist weit grösser (2-3 mal so gross als bei intraperitonealer) und die Reaktion verläuft etwas unregelmässig.

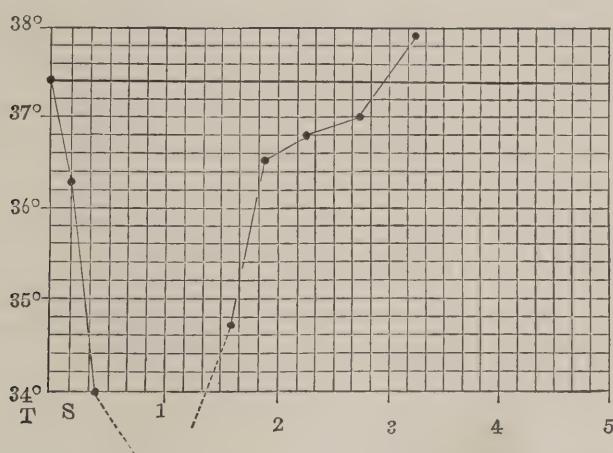


Fig. 4. Temperaturkurve.  
Nr. 227. 50,0 mg Rohaskaron in 3 ccm Kochsalzlösung subkutan.

TABELLE XI.

Datum.	Nummer.	Gewicht g.	Material.	Dosis g.	Resultat.
30/VII/15	24	220	Entfettetes Pulver.	0,75	stark
"	25	260	"	1,25	sehr stark
24/VIII	102	715	"	0,05	negativ
28/IX	130	200	Rohaskaron IV.	0,001	schwach
21/X	137	210	"	0,01	"
24/III/'16	230	120	Rohaskaron VI.	0,025	mittel
25/III	227	135	"	0,05	stark
"	245	460	"	0,1	sehr stark

#### 5. Einverleibung per os.

Bei dieser Applikation ruft das Askaron keine Reaktion hervor.

TABELLE XII.

Datum.	Nummer.	Gewicht g.	Material.	Dosis g.	Resultat.
6/VIII/'15	74	423	Rohpulver	0,4	negativ
1/IX	103	390	"	5,0	"
"	104	430	"	5,0	"

## 6. Lokale Reaktion.

Bei Meerschweinchen gibt es keine Augenreaktion und auch keine lokale Reaktion bei subkutaner oder intraperitonealer Injektion.

## c. Hunde.

Hunde sind widerstandsfähiger gegen das Askaron als Pferde und Meerschweinchen. Die individuelle Empfindlichkeit ist auch sehr divergent. Die Hauptsymptome bei der Vergiftung sind Erbrechen, Defäkation, Vermehrung der Absonderungen, Dyspnoe, Körperzittern, Krämpfe, Depression, Parese, Temperatursenkung und Herzschwäche; der Verlauf ist auch hier so schnell, dass die allgemeinen Symptome in 1-3 Stunden verschwinden. Selten gibt es Fälle, wo Tiere mit Enteritis und Herzleiden nach einer Erkrankung von 1-2 Tagen zu Grunde gehen. Autoptische Befunde zeigen Magen- und Darmblutung, Trübung von parenchymatösen Organen und Verzögerung der Blutgerinnung.

## BEISPIEL.

Nr. 1. Hündin, 5 Jahre alt. Gewicht 11,7 kg.

Stunde.	P.	A.	T.	Symptome.
2° 15'	130	12	37,8	Herztöne unregelmässig.
19	-	-	-	0,1 Rohpulver intravenös.
20	klein	45	39,1	Reflexerregung.
20-30	-	-	-	Erbrechen, Harnlassen, Defäkation, Salivation, nicht mehr aufstehen können, Rülpsen, Cyanose d. Konjunktiven, Erschlaffung der Sphinkter ani.
30	240	31	39,1	Senkung d. Hauttemperatur. Salivation, Nasenschleimfluss, Dyspnoe, Entkräftigung.

Stunde.	P.	A.	T.	Symptome.
40	232	38	36,9	Koma, Zuckung.
50	klein	24	36,9	
3° 10'	"	22	-	
20	192	26	36,9	
30	201	24	-	Erwacht, Salivation.
40	-	-	-	Zuweilen den Kopf aufhebt.
4° 0'	210	22	37,1	
45	-	-	-	Kotabsatz, Paralyse v. Rectum.
5° 30'	183	20	37,6	Kotentleerung (Blutig weich, mit stinkendem Geruch).
50	-	-	-	Atemstillstand (mittels künstlicher Atmung etwas erholt.)
7° 10'	212	26	38,4	Entkräftigung, Kolik.
5° 10'/18	-	-	-	Tot gefunden.

Befunde. Darmblutung (an Ileum deutlich.), Blutstauung v. Magen und Endkard, unvollkommene Blutgerinnung, Trübschwellung v. Leber u. Nieren.

TABELLE XIII.  
Intravenöse Injektion bei Hunden.

Datum.	Nummer.	Gewicht. kg	Material.	Dosis. g	Resultat.
17/II/15	7	17,0	Rohpulver (A. m)	0,1	Exitus
9/III	3	9,3	"	0,5	stark
"	4	4,1	"	0,3	schwach
9/III	6	9,3	"	0,5	"
"	7	6,3	"	"	"
"	8	5,0	"	"	mittel
22/III	9	6,5	" (A. l)	"	negativ
23/III	12	14,0	Rohaskaron IV.	0,02	stark
20/I/16	13	10,0	"	"	"
"	14	7,6	"	"	schwach
"	15	6,5	"	"	sehr stark
21/I	17	2,6	"	0,01	stark
"	18	2,5	"	"	schwach

## d. Kaninchen.

Kaninchen haben starke Resistenz gegen das Askaron und der individuelle Unterschied von Empfindlichkeit ist sehr gross. Die Hauptsymptome bei intravenöser Injektion sind Dyspnoe, Krampf, Kotabsetz und Depression. Bei schwerer Vergiftung verendet das Tier in einigen Minuten, aber wenn es die Vergiftung übersteht, erholt es sich in ungefähr 30 Minuten. Sektionsbefund ist Lungenblähung in leichtem Grade.

## BEISPIEL.

Nummer 13. Gewicht 2440 g.

Stunde.	Symptome.
3° 7'	5,0 mg Rohaskaron intravenös.
7-8	Dyspnoe, Stürzen, Krämpfe.
10	Schreien, heftige Krämpfe, Atemstillstand.
11	Exophthalmus, Exitus.

Befund: Leichte Lungenblähung.

Letale Dosis.

Wir können sie als 10 mg pro Kopf (d. i. 5 mg pro kg Tier) schätzen.

TABELLE XIV.

## Intravenöse Injektion.

Datum.	Nummer.	Gewicht. g	Material.	Dosis. g	Resultat.
1/III/'15	1	970	Rohpulver (A.m)	0,005	schwach
2/III	2	895	"	0,01	negativ
3/III	3	1040	"	0,015	"
"	4	990	"	"	"
6/III	5	860	"	0,05	schwach
20/X	6	1080	Rohaskaron IV	0,05	stark
25/X	7	1065	"	0,01	negativ
30/X	8	1435	"	0,005	sehr stark
"	9	1195	"	0,01	Exitus
14/I/'16	10	980	"	0,005	negativ
18/I	11	1580	"	0,003	mittel
21/I	12	2175	"	0,0025	negativ
"	13	2440	"	0,005	Exitus

## e. Ratten und Mäuse

Ratten und Mäuse sind stark resistent. Mit grosser Menge Askaron, z. B. 10 mg für Ratten intravenös und 5 mg für Mäuse subkutan einverleibt, kann man bei ihnen keine Reaktion herbeiführen.

2. WIRKUNG VON TOXISCHEN BESTANDTEILEN DER ANDEREN  
HELMINTHEN AUF MEERSCHWEINCHEN.

Ausser den Askariden (*Asc. lumbricoides et megalocephala*) gibt es verschiedene Helminthen wie *Sclerostomum equinum et vulgare*, *Filaria immitis*, *Trichocephalus depressiusculus*, *Oxyuris curvula* und auch Larven von *Gastrophilus equi*, deren giftige Bestandteile bei Meerschweinchen stark giftig wirken und dieselben Symptome und anatomische Veränderungen wie bei der Askaronvergiftung hervorrufen. Unsere Resultate stimmen mit den Ergebnissen überein, welche VAULLEGEARD mit *Taenia serrata* und deren *Cysticercus*, *Botholiocephalus punctatus*, *Moniezia neumanni*, und OTA und DEGUCHI mit Gastrophiluslarven, *Anoplocephala perfoliata*, *Filaria papillosa*, und *Sclerostoma vulgare et edentatum* bei Pferde konstatierten. Die letalen Dosen schwanken zwischen 0,01 und 0,02 g Rohpulver für Meerschweinchen. Gastrophiluslarvenpulver ist aber etwas arm an giftiger Substanz und es braucht eine 5 fache Menge von Askarispuvel um ein Meerschweinchen zu töten. Hier sind einige Extrakte aus dem Protokolle gegeben.

## BEISPIEL 1.

Nr. 139. Gewicht 190 g. (Den 8. Oktober 1915).

Stunde	Symptome.
2°10'	Temperatur 39,5. Normal.
12	0,02 g Rohpulver v. <i>Sclerostomum</i> intravenös.
13	Juckreiz, Würgebewegung, Atmung krampfhaft, Krämpfe, Husten, Krampfanfall.
14	Stürzt nieder, steht wieder auf; Schwanken, Krämpfe.
15	Seitenlage, Krämpfe, Atemstillstand.
16	Agoniale Atmung.
18	Herzschlag nicht mehr fühlbar, Exitus.

Sektionsbefunde: Hohe Lungenblähung, Blutgerinnung unvollkommen.

## BEISPIEL 2.

Nr. 136. Gewicht 265 g. (Den 8. Oktober 1915).

Stunde.	Temperatur.	Symptome.
3° 0'	39,8	Normal.
1	—	0,02 g Rohpulver v. <i>Oxyuris curvula</i> intravenös.
2	—	Erregt, Dyspnoe, Schütteln des Körpers, Kotentleerung, Krampfartige Atmung, Hustenanfall, Schwanken, Krämpfe.
4	—	Stürzt nieder; Krämpfe, Atemstillstand, Krämpfe. Atmung kehrt wieder.
5	—	Steht auf, Atemzüge immer zahlreicher, einige Schritte.
6	—	Atmung noch krampfhaft.
10	—	Depression, Pelz sich sträubend.
17	39,2	
32	38,8	Entkräftigung, Kaubewegung.
47	39,0	
4° 2'	39,0	Zittern.
17	39,4	
32	39,4	
47	39,6	
5° 27'	39,7	Erholt.

## BEISPIEL 3.

Nr. 142. Gewicht 190 g. (Den 9. Oktober 1915).

Stunde.	Symptome.
1° 55'	Temperatur 38,2° C. Normal.
58	0,01 g Rohpulver v. <i>Filaria immitis</i> intravenös.
59	Dyspnoe, Erregung, Erschütterung, krampfartige Atmung, Krampfanfall, schwankend.
2° 0'	Stürzt auf die Seite, Krämpfe.
1	Atem steht still, kehrt wieder, sistiert abermals, Krampf.
2	Agonale Atmung.
4	Herzschlag nicht fühlbar, Exitus.

Sektionsbefunde: Lungenblähung, epikardiale Ekchymosen, unvollkommene Blutgerinnung.

## BEISPIEL 4.

Nr. 296. Gewicht 170 g. (Den 31. März 1916).

Stunde.	Symtome.
4° 27'	Temp. 38,5° C.
30	Askaronfraktion aus 0,5 g Gastrophiluslarvenpulver intravenös.
31	Erregt, Dyspnoe, Krämpfe, Atmung auch krampfhaft, stürzt nieder, Krämpfe, Atmung sistiert.
32	Agoniale Atmung.
33	Exitus.

Sektionsbefunde : Lungenstarre, Gerinnung des Blutes unvollkommen.

TABELLE XV.

Intravenöse Injektion von Extrakten aus verschiedenen Helminthen.

Datum.	Nummer der Meer- schweinchen.	Körpergewicht g	Material.	Dosis. g	Resultat.
6/III'15	—	300	Rohpulver v. <i>Asc. megal.</i>	0,025	Exitus
6/III	—	285	"	0,006	stark
25/III'16	217	120	Rohpulver v. <i>Asc. lumb</i> aus Menschen.	0,01	sehr stark
8/III'15	137	205	Rohpulver v. Gastrophilus-larven.	0,01	negativ
"	138	"	"	0,02	"
29/III	165	225	"	0,05	"
"	166	180	"	0,01	"
31/III'16	269	170	Rohaskaron aus Gastrophiluslarven.	0,5	Exitus
8/III	139	190	Rohpulver v. Sclerostomen.	0,02	"
"	140	200	"	0,01	stark
1/IV'16	280	155	"	0,01	"
"	281	140	"	"	Exitus
"	280	150	"	0,006	"
"	278	140	"	0,005	"
"	277	150	"	0,0025	"
3/V	318	200	ditto. entfettetes Pulver.	0,0025	"
"	320	275	"	0,001	"

Datum.	Nummer der Meerschweinchen.	Körpergewicht g	Material.	Dosis. g	Resultat.
3/V	316	220	ditt. entfettetes Pulver.	0,001	sehr stark
"	317	248	"	0,0008	mittel
"	322	205	"	"	stark
9/III '15	142	190	Rohpulver v. Filarien.	0,01	Exitus
1/IV '16	283	160	"	0,005	mittel
"	285	160	"	0,01	Exitus
"	284	160	"	0,005	"
8/III '15	141	205	Rohpulver v. Distomen.	0,02	negativ
9/III	143	195	Rohpulver v. Taenien.	0,01	"
8/III	136	265	Rohpulver v. Oxyuris.	0,02	sehr stark
16/II '15	Hund	2400	Rohpulver v. Trichocephalus.	0,1	mittel

### 3. RESISTENZ NACH ASKARONVERGIFTUNG.

Die Tiere, welche die Vergiftung überstanden haben, werden hoch resistent gegen nachfolgende Injektionen von Askaron. Die Resistenz entsteht sofort nach der Injektion, erreicht am nächsten Tage ihr Maximum und nimmt allmählich ab, um in 1–2 Monaten ganz zu verschwinden. Nach der ersten intravenösen Injektion übersteht das Meerschweinchen schon in 4 Stunden eine 5 fache Menge, und am nächsten Tage eine 20 fache; nach einer Woche kann es nur einer 2 fachen resistieren. Bei der ersten intraperitonealen oder subkutanen Behandlung wird es noch resisternter und zwar übersteht es nach einem eintägigen Intervalle das 50–100 fache der sonst letalen Menge. Im Allgemeinen hängt der Resistenzgrad von der Reaktionsintensität und der Giftmenge bei der letzten Injektion und auch von der Individualität des Tieres ab.

Die Reaktion bei der zweiten Injektion verläuft wie bei der subkutanen Behandlung meist chronisch. In leichten Fällen steigt die Rektaltemperatur nach einer flüchtigen Abnahme um einige Zehntelgrade über die normale und kehrt dann ziemlich schnell wieder zurück.

## BEISPIEL 1.

Meerschweinchen Nr. 14. Körpergewicht 295 g (Den 26. Juni 1915).

Stunde.	Temp.	Symptome.
3° 33'	38,6	Normal.
40	—	1 mg entfettetes Pulver v. Askariden intrav., Dyspnoe.
45	—	Sträuben des Pelzes, Kotabsatz.
55	38,1	
4° 10'	37,8	Depression.
35	38,4	
50	38,7	Erholt.
6° 3'	39,1	
7	—	2,5 mg entfettetes Askaridenpulver (L.D.) intrav.
7-10	—	Husten, Dyspnoe, Rasseln, Erregung, Anfälle von Husten.
20	38,1	
35	38,4	
50	38,7	
7° 15'	38,7	
30	39,0	Alle Symptome verschwinden.
8° 0'	39,2	

## BEISPIEL 2.

Meerschweinchen Nr. 249. Körpergewicht 130 g (Den 30. März 1916).

Stunde.	Temperatur.	Symptome.
10° 45'	37,5	Normal.
48	—	0,35 mg Rohaskaron intravenös.
11° 4'	—	Kaubewegung, Kratzen des Gesichtes, Dyspnoe, Krampfanfall, es stürzt nieder, steht wieder auf, Atmung krampfhaft, Husten, Paralyse.
5	unter 34,0	Noch Krämpfe, Atemfrequenz grösser.
20	„	Entkräftigung, Depression
12° 5'	„	Zittern, Pelz sträubt sich.
35	„	
2° 40'	„	Das Tier wird etwas munterer.
4° 40'	37,0	
5° 40'	38,2	

Stunde.	Temperatur.	Symptome.
(Den 31. März.)		
7° 30' p.m.	38,0	Normal.
32-44	—	8,0 mg Rohaskaron (d. h. 25 fache v. L.D.) intrav. Erregt, krampfhaftes Husten, Dyspnoe, Krämpfe, legt sich, Parese der Hinterbeine.
45	35,4	Parese, Muskelatonie, Krämpfe, Atemstillstand: er kehrt wieder.
8° 0'	unter 34,0	Krampf, Depression, Zittern, Sträuben des Pelzes.
15	„	Krampf, Atemfrequenz grösser.
30	„	Entkräftigung, Zittern, Kotabsatz.
9° 0'	35,1	
30	35,8	Kaubewegung, Atmung beschleunigt.
10° 0'	37,1	
11° 0'	38,3	Erholt.
(Den 1. April)		
8° 5' a.m.	38,1	

## BEISPIEL 3.

Meerschweinchen Nr. 245. Gewicht 460 g (Den 25. März '16).

Stunde.	Temperatur.	Symptome.
10° 50' a.m.	38,5	Normal.
33-55	—	100,0 mg Rohaskaron subkutan. Kaubewegung, Kratzen, Dyspnoe, Husten, Atmung bedeutend schwer.
57	—	Kotabsatz, Schüttelung, Kaubewegung, Harnlassen, legt sich. Parese der Hinterbeine, Kopf herabgesenkt, es steht auf, legt sich wieder.
11° 2'	36,7	
5	—	Husten, es legt sich, Muskel erschlafft.
6	—	Kot, Dyspnoe noch dauernd.
15	—	Steht auf.
17	35,1	Kotabsatz, Depression.
22	—	Legt sich, noch dyspnoetisch.
30	—	Steht auf, Dyspnoe.
35	unter 34,0	

Stunde.	Temperatur.	Symptome.
54	34,2	Zittern.
12° 15'	unter 34,0	Entkräftigung, Zittern.
45	34,9	
1° 15'	35,0	
50	35,9	Etwas munterer, es geht.
2° 20'	36,7	
25	38,5	
55	38,0	
(26/März.)		
2° 10' p.m.	38,2	Das Tier etwas ermüdet.
14-20		50,0 mg Rohaskaron (125 fache v. L. D.) intravenös. Erregt, krampfhaftes Husten, Dyspnoe, leichter Krampf.
21-25		Atmung krampfhaft, Anfall von Husten, Zuckung, Würgbewegung, Schwanken.
26		Legt sich, noch dyspnoetisch, Kot, Parese der Hinterbeine.
30	36,6	Parese der Hinterbeine, Zuckung.
45	35,4	Dyspnoe.
3° 0'	35,2	Atmung beschleunigt, Zuckung, Depression.
30	35,2	Zittern.
4° 0'	36,2	Depression, Zittern.
30	36,3	
5° 30'	36,4	
6° 30'	37,2	Etwas munterer.
7° 30'	38,4	Erholt.
(27/März.)		
8° 35' a.m.	38,6	Ermüdet.

TABELLE  
Resistenzversuche bei

Datum.	Nr.	Gewicht. g	Material.	Dosis. mg
29/VI '15	13	265	entfett. Pulver	0,5
"	12	"	"	1,0
"	14	295	"	"
21	3	355	"	2,0
31/III '16	266	170	Rohaskaron VI.	0,4
"	267	"	ditto, mit Tierkohle behandelt	8,0
30	263	165	"	1,0
"	264	"	"	4,0
"	262	160	ditto, mit Na OH behandelt	0,4
"	211	170	ditto, bei 130° gekocht	0,25
"	267	160	ditto, mit HCl behandelt	0,5
"	258	"	"	1,0
"	256	180	Rohaskaron VI	0,25
"	248	150	"	0,4
"	249	130	"	0,35
21/VI '15	1	300	entfett. Pulver	1,0
"	2	255	"	2,0
23/	6	260	"	0,5
"	7	"	"	1,0
12/VIII	77	290	Rohaskaron I	0,04
25/III '16	217	120	Rohpulver	10,0
29/VI '15	21	250	entfett. Pulver	250,0 (p)
"	22	275	"	125,0 (p)
"	23	285	"	500,0 (p)
25/III '16	216	138	Rohaskaron VI	2,5 (p)
30/VI '15	24	220	entfett. Pulver	750,0 (c)
"	25	260	"	1250,0 (c)
25/III '16	227	135	Rohaskaron VI	50,0 (c)
"	245	460	"	100,0 (c)
14/VIII '15	81	240	Rohaskaron II	0,066
24/III '16	230	120	Rohaskaron VI	25,0 (c)
2/VII '15	17	340	entfett. Pulver	1,0*1
"	18	290	"	"

\* Letale Dosis v. entfett. Pulver = 2,5 mg, Rohaskaron III = 0,56 mg, Rohaskaron II, IV, subkutane Vorinjektion. (p) intraperitoneal, (c) subkutan.

## XVI.\*

## Meerschweinchen.

Resultat.	Intervall.	Material.	Dosis. mg	Resultat.
schwach	2° 30'	entfett. Pulver	2,5	schwach
"	"	"	"	mittel
"	"	"	"	schwach
stark	4° —	"	2,0	"
sehr stark	4° 30'	Rohaskaron IV	4,0	"
stark	"	"	2,0	mittel
negativ	1 Tage	"	0,8	schwach
"	"	"	2,0	Exitus
schwach	"	"	"	"
"	"	"	"	"
mittel	"	"	"	sehr stark
"	"	"	"	schwach
sehr stark	"	"	4,0	"
stark	"	"	8,0	Exitus
sehr stark	"	"	"	sehr stark
stark	"	entfett. Pulver	4,0	negativ
"	"	"	"	"
negativ	"	"	5,0	"
stark	"	"	"	"
schwach	"	"	"	"
sehr stark	"	Rohaskaron VI	"	schwach
"	"	entfett. Pulver	2,5	negativ
schwach	"	"	"	"
mittel	"	"	"	"
"	"	Rohaskaron VI	20,0	schwach
stark	"	entfett. Pulver	50,0	negativ
sehr stark	"	"	85,0	"
stark	"	Rohaskaron VI	5,0	"
sehr stark	"	"	50,0	stark
schwach	2 Tage	entfett. Pulver	5,0	negativ
stark	"	Rohaskaron VI	20,0	Exitus
negativ	"	entfett. Pulver	10,0	"
"	"	"	"	mittel

VI=0,4 mg  $\times 1$ ,  $\times 2$  bedeuten die Zahl v. intravenösen Injektion vor dem Versuche,  $\times 1s$  die

Datum.	Nr.	Gewicht. g	Material.	Dosis. mg
2/VII '15	19	270	entfett. Pulver	1,0×1
"	20	225	"	"
24/VI	6	260	"	5,0×1
"	7	250	"	"
2/IX	102	705	"	500,0×2(p)
24/VI	9	220	"	3,0
"	10	190	"	1,0
22/II '16	189	230	Rohaskaron IV	0,2
"	192	267	"	"
"	193	235	"	"
"	194	220	"	0,4
27/VI '15	12	265	entfett. Pulver	2,5×1
"	14	295	"	"
24	1	300	"	20,0×3
"	2	255	"	"
"	3	355	"	"
26/VII	33	290	"	2,5×2
14	"	280	"	5,0×1
17/VIII	84	253	"	1,0
"	87	147	"	0,75
21	89	255	"	2,5×1
4/IX	34	440	"	2,5×2
14/VII	31	310	"	10,0×1
16/VIII	81	240	"	5,0×1
1/VII	21	230	"	25,0×2
"	23	300	"	100,0×3
"	22	255	"	50,0×2
2/VIII	"	430	"	5,0×3
4/IX	"	440	"	2,5×4
28/VII	66	225–380	"	5,0×1
21	49	240–325	"	2,5×1
"	53	245–357	"	5,0×1
"	54	225–380	"	"
"	55	220–265	"	10,0×1
"	56	220–280	"	5,0×1
14	34	220–410	"	10,0×1
1	24	220–392	"	50,0×18
"	25	265–470	"	80,0×18

Resultat.	Intervall.	Material,	Dosis. mg.	Resultat.
schwach	2 Tage	entfett. Pulver	10,0	mittel
negativ	"	"	"	"
"	"	"	20,0	negativ
"	"	"	"	"
"	"	"	50,0	Exitus
"	3 Tage	"	5,0	"
"	"	"	"	"
stark	"	Rohaskaron VI	5,0	schwach
schwach	"	"	"	Exitus
mittel	"	"	"	"
stark	"	"	"	negativ
mittel	5 Tage	entfett. Pulver	2,5	Exitus
schwach	"	"	"	"
negativ	6 Tage	"	50,0	"
"	"	"	"	"
"	"	"	25,0	"
schwach	7 Tage	"	5,0	"
negativ	12 Tage	"	2,5	schwach
mittel	14 Tage	"	"	"
negativ	"	"	"	negativ
schwach	"	"	"	Exitus
stark	"	Rohaskaron III	1,0	"
negativ	18 Tage	entfett. Pulver	5,0	"
"	19 Tage	"	2,5	negativ
stark	25 Tage	"	"	Exitus
mittel	"	"	"	schwach
schwach	32 Tage	"	5,0	"
"	33 Tage	"	2,5	sehr stark
sehr stark	"	Rohaskaron IV	0,1	Exitus
negativ	38 Tage	entfett. Pulver	2,5	schwach
schwach	43 Tage	"	"	stark
mittel	"	"	"	Exitus
negativ	"	"	"	"
schwach	"	"	"	schwach
"	"	"	"	"
negativ	52 Tage	"	"	stark
"	60 Tage	"	5,0	Exitus
"	"	"	2,5	schwach

TABELLE XVII.

Resistenzversuche bei Meerschweinchen.

(Zum erstenmal per os behandelt.)

Nummer.	Gewicht g.	Intervall Tag.	Material.	Dosis g.	Resultat.
74	423	—	Rohpulver v. asc. lumb	0,4 (p. o)	negativ
"	"	1	"	" ( „ )	"
"	"	1	"	" ( „ )	"
"	"	1	"	" ( „ )	"
"	"	1	entfettetes Pulver	0,005 (v)	exitus
103	390	—	Rohpulver	5,0 (p. o)	negativ
"	"	1	entfettetes Pulver	0,003 (v)	sehr stark
104	430	—	Rohpulver	5,0 (p. o)	negativ
"	"	1	entfettetes Pulver	0,005 (v)	stark

TABELLE XVIII.

Resistenzversuch bei Pferden.

Namen.	Injektions-nummer.	Intervall, Tag	Material.	Dosis, g	Resultat.
Mikadzuki	1.	—	Rohpulver (A. m.)	0,1 (v)	negativ
	2.	1	"	0,5 "	stark
	3.	5	"	0,54 "	sehr stark
	4.	12	Rohpulver (A. l.)	0,5 "	stark
	5.	60	"	0,5 "	Exitus
Yuwane	1.	—	Rohaskaron II	0,0002 (c)	sehr stark
	2.	77	" (IV)	0,0001 "	negativ
	3.	26	"	0,0002 "	"
	4.	12	"	0,0005 "	"
	5.	7	"	0,001 "	"
	6.	2	"	0,005 "	"
Shuntei	1.	—	"	0,0001 "	mittel
	2.	2	"	0,001 "	"
	3.	30	"	"	negativ
	4.	2	"	0,005 "	"
Shichitei	1.	—	"	0,0001 "	schwach
	2.	40 Minuten	"	" (v)	stark
Shiki	1.	—	"	0,00008 "	schwach

Namen.	Injektions-nummer.	Intervall. Tag	Material.	Dosis. g	Resultat.
Shinagawa	2.	25 Minuten	Rohaskaron (IV)	0,0003 (c)	negativ
	1.	—	"	0,0005 (v)	stark
	2.	3	"	0,01 "	Exitus

TABELLE XIX.  
Resistenzversuch mit Rohaskaron IV bei Hunden.

Nummer.	Intervall. Tag	Dosis. mg	Resultat.
12	—	20,0 (intrav.)	stark
	1	"	negativ
13	—	"	stark
	1	40,0	"
14	—	20,0	schwach
	1	40,0	negativ
15	—	20,0	sehr stark
	1	40,0	negativ

Die Resistenz entsteht nicht nur bei Askaronvergiftung sondern auch bei Vergiftung durch Bestandteile von verschiedenen anderen Helminthen. Die Schnelligkeit ihrer Entstehung, ihre Stärke und ihre Dauer stimmen mit der Askaronresistenz ganz überein. Die durch die verschiedenen Gifte erlangten Resistenzen wirken in jeder Hinsicht gegenseitig. So schützt z. B. Sclerostomenvergiftung das Tier gegen nachfolgende Askaroninjektion.

#### BEISPIEL 1.

Meerschweinchen 316. Gewicht 220 g. (Den 3. Mai 1916.)

Stunde.	Temp.	Symptome.
3° 5'	38,4	Normal.
7-10	—	1,0 mg entfettetes Sclerostomenpulver intravenös. Erregt, Schreien, Kratzen, Husten, Atmung krampfhaft, es schwankt.
11	—	Legt sich auf die Seite, Anfall von Krämpfen.
17	—	Atemfrequenz grösser.

Stunde.	Temp.	Symptome.
20	—	Steht auf, Depression, Pelz sträubt sich.
22	37,0	
37	35,7	
52	35,3	Kratzen, Entkräftigung, noch dyspnoetisch.
4° 7'	35,2	
22	35,6	Zittern, Depression.
37	36,5	
52	37,0	
5° 7'	37,3	
22	37,5	
37	37,6	Atmung normal.
52	37,9	
6° 7'	37,8	Etwas munterer.
22	37,6	
37	38,5	Erholt.
(4/Mai.)		
5° 24'	38,5	Keine Veränderung.
32-35	—	6,0 mg Rohaskaron (d. i. 10 fach v. L. D.) intravenös. Dyspnoe, Würgebewegung, Kratzen, krampfhaft Atmung, Krampf, schwankt, legt sich.
36	—	Steht auf, Atmung stärker, leichter Krampf.
40	—	Depression, Sträuben des Pelzes, Zittern.
47	38,0	Ermüdet, Kratzen.
6° 0'	37,2	Etwas munterer.
7° 0'	38,2	
8° 0'	38,7	

## BEISPIEL 2.

Meerschweinchen 322. Gewicht 240 g. (Den 4. Mai 1916.)

Stunde.	Temp.	Symptome.
4° 42'	38,2	Normal.
46	—	0,4 mg Rohaskaron intravenös.
47	—	Kaubewegung, unruhig, erregt, Anfall von Husten, Krämpfe, Dyspnoe.

Stunde.	Temp.	Symptome.
48	—	Es schwankt, stürzt nieder, Seitenlage, es folgen heftige Krämpfe, Atmung minder und lang. Dyspnoe.
52	—	Steht auf.
57	36,5	Parese, Zuckung der Hinterbeine.
5° 12'	35,1	Atemfrequenz vermehrend. Depression.
42	36,4	Atmung normal, Haare sträuben sich.
6° 12'	36,6	
7° 12'	37,9	Munterer.
8° 12'	38,5	Erholt.
(5/Mai.)		
3° 15'	38,0	Normal.
18	—	0,01 g Sclerostomenpulver (d. i. 10 fach v. L. D.) intravenös.
20		Zuckung, leicht dyspnoetisch, Depression.
33	37,6	
4° 33'	38,1	
5° 33'	38,9	

TABELLE XX.

## Gemeinresistenz.

Datum.	Nr.	Gewicht. g	Material.	Dosis. mg	Resultat.	Intervall. Tag	Material.	Dosis. mg	Resultat.
25/III'16	217	120	Rohpulver (A. l. aus Menschen)	0,01	sehr stark	1	Rohaskaron VI	5,0	schwach
8/X'15	140	200	,, v. Sclerostomen	„	stark	„	„ IV	0,2	negativ
„	136	265	Oxyurispulver	0,02	sehr stark	„	„	0,4	„
1/IV'16	283	160	Filarienpulver	0,005	mittel	„	„	2,0	stark
3/V	316	220	entfett. Pulv. v. Sclerostomen	0,001	sehr stark	„	„	6,0	mittel
„	317	248	„	0,0008	mittel	„	„	2,0	sehr stark
„	322	205	„	„	stark	„	„	4,0	„
„	321	240	Rohaskaron IV	0,0004	sehr stark	„	entfett. Pulv. v. Sclerostomen	10,0	negativ
„	323	180	„ VI	0,0005	schwach	„	„	10,0	„

Aus den oben erwähnten Resultaten ergibt sich folgendes Resumé.

1. Das Askaron ruft bei Pferden, Hunden, Kaninchen und Meerschweinchen Symptome und Sektionsbefunde hervor, die ähnlich sind denen beim anaphylaktischen Shocke.

2. Pferde, Meerschweinchen, Hunde und Kaninchen sind absteigenderweise empfindlich gegen Askaron, und Ratten und Mäuse so resistent, dass man bei ihnen selbst mit einer grossen Giftdosis keine Reaktion herbeiführen kann.

3. Die letale Dosis von Askaron bei intravenöser Injektion ist bei Pferden 1,0 mg (0,004 mg pro kg Tier), bei Meerschweinchen 0,2–0,4 mg (0,8 mg pro kg Tier), bei Hunden 20,0 mg (2,0 mg pro kg) und bei Kaninchen 5–10,0 mg (5,0 mg pro kg).

4. Die Giftigkeit von Askaron ist sehr divergent je nach der Applikationsweise; bei subkutaner Injektion ist die Dosis bedeutend grösser als bei intravenöser, und bei derjenigen per os ist das Gift wirkungslos.

5. Die Veränderungen (wie Augenreaktion, nervöse Störung, Erweiterung von Kapillargefäßen und Veränderung des Blutes), welche FLURY den zahlreichen giftigen Fraktionen von Askariden zuschreibt, können ganz sicher mit Robaskaron herbeigeführt werden, obgleich dieses keine der von FLURY erwähnten Substanzen enthält.

6. Aus Mangel an Materialien haben wir die giftigen Bestandteile von verschiedenen Helminthen noch nicht genau untersucht. Aus der Tatsache, dass die Symptome bei den Vergiftungen von verschiedenen Helminthen dieselben sind, dass die Resistenzwirkung gegenseitig ist, und dass die wirksamen Bestandteile immer eine Asakaronfraktion enthalten, glauben wir schliessen zu können, dass die toxische Substanz von anderen verschiedenen Helminthen mit Askaron identisch sei.

#### **IV. Differenzierung der Askaronvergiftung von dem anaphylaktischen Shocke.**

Während die Ähnlichkeit der Symptome mit denen des anaphylaktischen Shockes und die schnelle Entstehung von hoher Resistenz in der Askaronvergiftung einen anaphylaktischen Vorgang erblicken lassen, ist aber doch

ein grosser Unterschied zwischen den beiden, dass das Askaron ohne Vorbehandlung schon bei der ersten Injektion toxisch ist. Hier müssen wir somit zuerst entscheiden, ob das normale, nicht behandelte Blut Antikörper gegen Wurmleibessubstanzen enthalte oder ob das Askaron primär toxisch sei. Wir wollen mit den folgenden Punkten die Anschauung verneinen, dass die Tiere durch die Substanz der schmarotzenden Würmer schon natürlich vorbehandelt wären.

1. Bei Meerschweinchen ist das Vorhandensein von Antikörpern gegen die Leibessubstanz der Helminthen, welche das Tier nicht invasierten, nicht denkbar, obgleich sie ihm eine schwere Vergiftung verursachen kann.
2. Das Askaron kann bei hinreichender Menge bei Pferden, Hunden, Kaninchen und Meerschweinchen ohne Ausnahme Vergiftung hervorrufen, und bei Meerschweinchen ist die individuelle Empfindlichkeit so konstant, dass die letale Dosis immer konstant ist.
3. Da die Resorption von gerinnbarer Eiweißsubstanz selbst aus dem Verdauungskanale normalerweise nicht denkbar ist, so ist die Voraussetzung unhaltbar, dass die Eiweißsubstanz von Magendarmschmarotzern ihren Wirten eine konstante Ueberempfindlichkeit verleihen könnte.
4. Das Askaron ist eine Substanz von Albumose-Peptonencharakter, welcher nicht als ein gutes Anaphylaktogen betrachtet wird.

#### 1. PRIMÄRE TOXIZITÄT VON ASKARON.

Um unsere Annahme zu prüfen, haben wir weiter bei Pferden die folgenden Versuche durchgeführt.

1. Versuch nach aktivo-passiver Anaphylaxie. Eine Reihe von Meerschweinchen wird mit 2–5 ccm normalem Pferdeblute intraperitoneal behandelt und ihnen nach einem 24 stündigen Intervalle  $1/5-1/8$  der letalen Dosis von Askaron intravenös injiziert. Eine Veränderung der Toxizität ist hier nicht wahrnehmbar, wie aus der folgenden Tabelle ersichtlich ist.

TABELLE XXI.

Datum.	Nummer.	Gewicht. g	Vorbehandlung mit Pferdeserum. ccm	Probe. g	Resultat.
2/VII'15	23	625	3,0	Rohpulv. v. A. 1. 0,001	schwach
"	24	620	"	"	negativ
"	25	690	2,0	"	"
"	26	470	"	"	"
5	29	720	7,0	"	"
7	30	270	3,0	"	"
"	31	"	"	0,0005	schwach
"	32	285	"	0,002	negativ
"	33	270	"	"	"
6/VIII'16	83	215	5,0	entfett. Pulv. v. A. 1. 0,0005	"
"	84	255	"	0,001	schwach
"	85	210	"	0,0005	negativ
"	86	222	"	"	"
"	87	250	"	0,00075	"

Bemerk.: L. D. v. Rohpulver 0,01 g, entfettetes Pulver 0,0025 g.

2. Sind entfettetes Askaridenpulver und Askaron als Anaphylaktogen wirksam? Bei der Vorbehandlung wird den Meerschweinchen 1/2-1/5 der letalen Dosis von entfettetem Askaridenpulver oder Rohaskaron subkutan injiziert, und nach einem 14 tägigen Intervalle wird 1/2-1/4 der letalen Dosis intravenös probiert. Eine Vermehrung der Toxizität ist aber nicht zu konstatieren.

TABELLE XXII.

(A) Material: entfettetes Pulver von *Ask. lumb.*

Datum.	Nummer.	Gewicht. g	Intervall. Tage	Dosis.		Resultat.
				Vorbehand. mg	Probe. mg	
27/VIII'15	77	213	14	0,005	0,00125	negativ
30	83	230-265	13	"	"	"
"	84	253-275	"	0,001	0,0025	"
"	85	215-257	"	0,0005	0,00125	"
"	86	240-287	"	"	"	schwach
"	87	247-277	"	0,00075	0,0025	negativ

(B) Material : Rohaskaron u. getrocknete Leibesflüssigkeit von *Ask. lumb.*

Datum.	Nummer.	Gewicht. g	Interval. Tage	Dosis.		Resultat.
				Vorbehand. mg	Probe. mg	
21/X/15	12	450	22	L.H.F. 0,1 (c)	Rohask., IV. 0,1	negativ
	42	410	"	"	0,2	Exitus
	66	310	"	0,2	0,1	negativ
27	148	580-535	14	Rohask., IV. (c) 0,1	"	"
	149	490-470	"		"	"
	150	705-435	"	"	0,2	stark
	151	375-335	"	"	"	"

3. Komplementablenkung. Die Komplementablenkungsversuche mit Rohaskaron als Antigen bei 17 Arten normaler Pferden und bei 5 Arten Schweinesera fielen alle negativ aus.

Wir haben zuerst festgestellt, dass eine 1%ige Rohaskaronlösung oder ein Gemisch von Rohaskaron und Komplement auf Ziegenblutkörperchen hämolytisch unwirksam ist, und dass das Rohaskaron in dem hämolytischen Systeme keine Störung ausübt.

a). Versuchsschemata sind folgende :

Inaktiviertes Pferdeserum ccm.	Rohaskaron mg.	Komplement ccm.	Ziegen-, Kanin- chenserum ccm.	Ziegenblutkö- rperchen.	R <sub>1</sub>	R <sub>2</sub>
0,5	0,4	0,1	0,002	1,0		
"	0,2	"	"	"		
"	0,1	"	"	"		
"	0,05	"	"	"		
"	0,025	"	"	"		
"	0,0125	"	"	"		
"	0,00625	"	"	"		
"	0,003125	"	"	"		
"	0,0015625	"	"	"		
"	0,0007812	"	"	"		
"	-	"	"	"	22 Stunden im Eisenschrank.	
"	-	"	"	"		
-	0,4	"	"	"		
-	"	"	"	"		

1 Stunde bei 37° C.

b).

Inaktiviertes Pferdeserum ccm.	Antigen Rohaskaron mg.	Komplement cm.	Ziegen-, Kaninchenserum ccm.	Ziegenblutkörperchen ccm.	R <sub>1</sub>	R <sub>2</sub>
0,5	0,1	0,1	0,001	1,0		
0,25	"	"	"	"		
0,125	"	"	"	"		
0,0625	"	"	"	"		
0,03125	"	"	"	"		
0,5	—	"	"	"		
"	—	"	"	"		
—	0,1	"	"	"		
—	"	"	"	"		

TABELLE XXIII.

Komplementablenkung mit Seren aus normalen Tieren und Rohaskaron.

Datum.	Nummer d. Tiere.	Inakt. Serum ccm.	Antigen (Rohaskaron) mg.	Titer v. Zieg. Kaninchenserum.	Resultat.	Bemerkung.
5/VII'15	Pferd 1	0,2	5,0-0,5	1:1000	negativ	Antigenverdünnung
"	2	0,5	"	"	"	"
12/X	3	"	0,4-0,04	"	"	"
15	4	"	0,4-0,003	"	"	"
9/XI	5	"	"	1:500	"	"
"	6	"	"	"	"	"
17/XII	7	"	0,4-0,05	1:1000	"	"
14/I'16	8	"	0,4-0,0008	1:500	"	"
"	9	"	"	"	"	"
"	10	"	"	"	"	"
"	Schwein 1	"	"	"	"	"
"	2	"	"	"	"	"
"	3	"	"	"	"	"
"	4	"	"	"	"	"
"	5	"	"	"	"	"
2/II	Pferd 11	0,25	0,4-0,0001	"	"	"
5/IV	12	0,5-0,03125	0,1	1:150	"	Serumverdünnung
"	13	"	"	"	"	"
"	14	"	"	"	"	"
"	15	"	"	"	"	"
2/V	16	"	"	1:1000	"	"
"	17	"	"	"	"	"

4. Präzipitinreaktion. Die Präzipitinreaktion fällt auch bei normalen Pferden ganz negativ aus. Versuchsschemata und Resultate sind folgende.

a).

Nr.	Serum	Rohaskaron.	R <sub>1</sub>	R <sub>2</sub>
1.	100%	1%		
2.	"	0,5		
3.	"	0,25		
4.	"	0,125		
5.	"	0,0625		
K <sub>1</sub>	"	1% Witte Pepton		
K <sub>2</sub>	"	-		
		30 Minuten bei 37° C.	2 Stunden bei 37° C.	

b).

Nr.	Serum	Rohaskaron.	R <sub>1</sub>	R <sub>2</sub>
1.	100%	0,14%		
2.	50	"		
3.	25	"		
4.	12,5	"		
5.	6,25	"		
K <sub>1</sub>	50,0	1% Witte Pepton		
K <sub>2</sub>	-	0,1% Rohask.		
		30 Minuten bei 37° C.	2 Stunden bei 37° C.	

TABELLE XXIV.

Präzipitinreaktion mit Seren aus normalen Pferden und Rohaskaron.

Datum.	Nummer d. Tiere.	Serum. %	Rohaskaron. %	Resultat.	Bemerkung.
13/X '15	1	100-6,25	0,1	negativ	Serumverdünnung
15/XI	2	"	"	"	"
19	3	"	"	"	"
22	4	"	"	±	"
"	5	"	"	±	"
"	6	"	"	"	"
"	7	"	"	"	"
"	8	"	"	"	"
29	9	"	"	negativ	"
"	10	"	"	"	"
6/XII	11	"	"	"	"
"	12	"	"	"	"
8	13	"	0,1 (L.H.F.)	"	"
14	14	"	0,1	"	"
"	15	"	"	"	"
14/I '16	16	"	"	"	"
"	17	"	"	"	"
"	18	"	"	"	"
5/IV	19	100	1-0,065	±	Antigenverdünnung
"	20	"	"	"	"
"	21	"	"	negativ	"
"	22	"	"	"	"
"	23	"	"	"	"

Hier haben wir das Vorhandensein von Antikörpern gegen das Askaron in normalen Seren zu verneinen und die primäre Toxizität von Rohaskaron als konstatiert zu betrachten. Wir wollen somit das Askaron mit FRIED-

BERGERSchem Anaphylatoxine oder mit anderen verwandten Substanzen weiter verglichen und differenzirt.

## 2. ASKARONRESISTENZ UND ANTIANAPHYLAXIE.

Unter den chemischen oder enzymatischen Spaltprodukten der Eiweissubstanzen sind mehrere organische Basen und auch einige höhere Eiweissspaltprodukte, welche der Albumose-Peptonengruppe angehören, als giftig wirksam bewiesen. Von diesen sind als solche, welche einem anaphylaktischen Shocke ähnliche Symptome hervorrufen, das Histamin ( $\beta$ -Imidazolyläthylamin), Methylguanidin und Peptonum Witte zu nennen.

Histaminvergiftung hinterlässt aber keine Resistenz gegen abermalige Injektion und dabei ist die Blutgerinnung meistens beschleunigt; und Methylguanidin kann erst mit 10,0 mg ein Meerschweinchen töten, während die letale Dosis von Rohaskaron nur 1/50 davon ist. Auch die wirksame Substanz von Peptonum Witte kann mit Askaron nicht identisch sein, weil das Meerschweinchen 20-30 mal weniger empfindlich ist als der Hund, während das erstere für das Askaron 4 mal empfindlicher ist. Das Kaninchen ist zwar für Peptonum Witte nicht empfindlich, für Askaron aber ist es sicher empfindlich. Ferner sind der Grad und die Dauer der Askaronresistenz weit grösser als diejenigen von Peptonschütze.

Die Anhänger der Anaphylatoxintheorie nehmen an, dass Anaphylatoxin ein peptonenartiges Reaktionsprodukt von Antigeneiweiss und Antikörper sei und sie wollen weiter giftige höhere Eiweissspaltprodukte, welche durch biologische Reaktion *in vitro*, Verdauung oder durch Säure- und Alkaliwirkung entstehen können, daran reihen. Da das Askaron in den chemischen Eigenschaften und Wirkungen dem Anaphylatoxin im erwähnten Sinne sehr ähnlich scheint, so haben wir eine Vergleichung ihrer Wirkungen und Derivierung von Askaron aus gerinnbarem Askarideneiweiss versucht. Gegenüber der Hitzbeständigkeit von Askaron ist das Anaphylatoxin *in vitro* thermolabil und es wird durch eine kurze Erhitzung bei 65°C zerstört. Wir haben somit, um die Wirkung von Anaphylatoxin *in vivo* mit Askaron zu vergleichen, den Gemeingrad von Serumantianaphylaxie und Askaronresistenz miteinander untersucht.

Eine Gruppe von mit normalem Pferdeserum vorbehandelten Meerschweinchen werden am Ende der Inkubation mit Rohaskaron schwer vergiftet und dann am nächsten Tage mit Serum probiert, und die andere am nächsten Tage nach der Serumprobe mit Rohaskaron behandelt.

TABELLE XXV. a.

(Vorbehandlung: 0,1 ccm Pferdeserum subkutan am 8. Oktober).

Nr.	Gewicht g.	Probe od. Ask. Injektion.			Ask. Injektion od. Probe.		
		Datum.	Dosis.	Resultat.	Datum.	Dosis.	Resultat.
106	345	22/X	Pferdeserum 0,2 ccm	Exitus	—	—	—
104	465	„	0,1	stark	23/X	Rohaskaron 4,0 mg	Exitus
105	240	21	1,0 (c)	schwach	22	1,0	„
108	305	{ 21	1,0 (c)	schwach	23	0,4	schwach
		{ 22	2,0 (p)	negativ			
110	320	{ 21	1,0 (c)	schwach	„	1,0	„
		{ 22	2,0 (p)	negativ			
138	310	22	1,0 (c)	schwach	22	0,4	mittel
141	175	{ 21	1,0 (c)	„	23	Pferdeserum 0,2 ccm	Exitus
		{ 22	2,0 (p)	negativ		Rohaskaron 0,2 mg	stark
(120)	280	—	—	—	22		
(135)	200	—	—	—	„	“	“
(164)	280	—	—	—	„	0,4	Exitus
137	210	21	Rohaskaron 10,0 mg (c)	schwach	„	Pferdeserum 0,2 ccm	“
107	300	{ 21	“	“	23	0,2	schwach
		{ 22	“ (p)	negativ			
109	330	{ 21	“ (c)	schwach	„	0,3	mittel
		{ 22	“ (p)	negativ			
103	400	{ 21	“ (c)	schwach	„	0,4	stark
		{ 22	“ (p)	negativ			

TABELLE XXV. b.

Vorbehandlung: Meerschweinchen wurden am 23. Oktober je mit  
0,01 ccm Pferdeserum subkutan injiziert.

Nr.	Gewicht g.	Probe.			Askaroninjektion.		
		Datum.	Dosis ccm.	Resultat.	Datum.	Dosis mg	Resultat.
85	290	4/XI	0,075	Exitus	—	—	—
95	380	„	“	“	—	—	—
114	215	„	0,05	“	—	—	—
84	210	„	“	mittel	5/X	P.S. 0,4 ccm	Exitus
39	432	„	0,1	schwach	„	Ask. 0,4 mg	“

Nr.	Gewicht g.	Probe.			Askaroninjektion.		
		Datum.	Dosis ccm.	Resultat.	Datum.	Dosis mg.	Resultat.
25	345	4/XI	0,05	schwach	5/X	Ask. 0,4 mg	Exitus
46	365	"	0,075	mittel	"	"	mittel
62	250	"	0,05	"	"	0,6	Exitus
117	205	"	"	"	"	"	"
60	275	"	"	schwach	"	0,4	negativ
56	260	"	"	sehr stark	"	0,6	stark
93	380	"	"	schwach	"	0,4	"
55	175	{ 4 5 (8° 22')	0,05 0,20	stark schwach	" (9°)	0,6	"
116	187	{ 4 5 (8° 30')	0,05 0,20	stark schwach	" (9° 30')	"	schwach
86	275	{ 4 5 (6° 40')	0,05 0,20	stark schwach	" (8° 38')	0,8	Exitus
112	300	{ 4 5 (7° 15')	0,05 0,30	schwach	" (8° 44')	1,0	"
92	250	{ 4 5 (7° 30')	0,05 0,40	stark "	" (8° 55')	1,0	stark

TABELLE XXV, c.

Subkutane Präparierung mit 0,01 ccm Pferdeserum (den 23. März).

Nr.	Gewicht g.	Probe od. Askaroninjektion.			Askaroninjektion od. Probe.		
		Datum.	Dosis.	Resultat.	Datum.	Dosis.	Resultat.
210	200	6/IV	Pferdeser. 0,05 ccm	Exitus	-	-	-
214	235	"	0,03	"	-	-	-
208	240	"	0,025	stark	7/IV	Rohaskaron 2,0 mg	mittel
209	250	"	"	"	"	3,0	Exitus
215	215	"	"	sehr stark	"	2,0	sehr stark
211	225	"	"	"	"	2,4	Exitus
218	230	"	"	"	"	"	sehr stark
(294)	180	"	Rohaskaron 0,3 mg	mittel	"	6,0	schwach
(295)	195	"	0,4	sehr stark	"	12,0	mittel
225	240	"	0,6	Exitus	"	-	-

Nr.	Gewicht g.	Probe od. Askaroninjektion.			Askaroninjektion od. Probe.		
		Datum.	Dosis.	Resultat.	Datum.	Dosis.	Resultat.
219	205	6/IV	0,5	mittel	7/IV	Pferdeser. 0,09 ccm	Exitus
220	270	"	0,3	schwach	"	"	sehr stark
221	205	"	"	"	"	"	"
222	195	"	0,4	mittel	"	0,12	stark
223	205	"	"	schwach	"	"	Exitus
224	215	"	"	"	"	"	"
226	265	"	0,5	"	"	0,06	"
228	250	"	"	mittel	"	"	"
229	190	"	"	schwach	"	"	Exitus

Die Gemeinresistenz von natürlichem Anaphylatoxin mit dem Askaron erreicht also nur 2–3 mal die letale Dosis und ist zu klein gegenüber der Askaronresistenz selbst, um eine Uebereinstimmung von beiden Resistzenzen zu zeigen. Dass die Askaronresistenz nach einer subkutanen Vorbehandlung mit grossen Dosen das 100 fache der letalen Dosis erreichen kann, und dass bei Pferden aktive Immunität gegen das 400 fache der letalen Dosis erreicht werden kann, ohne dass sich die Entstehung von humoralen nachweisen lässt, das alles zwingt uns hier anzunehmen, dass die Askaronresistenz aus zwei Faktoren, d.i. aus zellulär spezifischer und aspezifischer Resistenz bestehe. Da die Identität von Anaphylatoxin in vivo mit dem in vitro noch nicht bewiesen ist und der Umstand, dass künstliches Anaphylatoxin nur schwache aspezifische Resistenz (im Sinne FRIEDBERGERS) herbeiführt, nicht direkt auf Anaphylatoxin in vivo übertragbar ist, so kann man allerdings vermuten, dass das Askaron eine dem Anaphylatoxin in vivo angehörende Substanz und ihre Differenz nur in der Spezifität zu erblicken sei.

Mittels Autolyse und künstlicher Verdauung von gerinnbarem Askariden eiweiß wollten wir die Matrix von Askaron finden. Wir haben zuerst die Giftigkeit von gerinnbarem Eiweiß geprüft und dann diejenige von Autolysaten und Verdauungsprodukten. Die Leibeshöhlenflüssigkeit oder der wässrige Extrakt von der Leibessubstanz der Askariden wird mit Essigsäure schwach angesäuert und bei 100°C eine Stunde lang gekocht. Das Filtrat,

frei von geronnenem Eiweiss, büsst aber seine ursprüngliche Toxizität nicht ein. Bei der direkten Einverleibung sind wässriger Extrakt, Albuminfraktion und Globulinfraktion von den in strömendem Wasser gewaschenen und möglichst von Leibesflüssigkeit und Askaron befreiten Würmern noch toxisch und ihre letalen Dosen pro Kopf Meerschweinchen sind je 3,0 mg 6,0 mg und 3,0 mg resp. Obgleich die Beimengung von Askaron nicht ausgeschlossen ist, müssen wir doch die Giftigkeit von gerinnbarem Eiweiss annehmen.

TABELLE XXVI.

## Toxizität von gerinnbarem Askarideneiweiss.

Datum.	Nummer.	Gewicht g.	Material.	Dosis mg.	Resultat.
27/VII	65	250	wäss. Ext. v. frischen Askariden	2,5	schwach
"	66	230	"	5,0	stark
"	68	240	"	"	sehr stark
"	63	250	ditto, mit Essig angesäuert u. enteiweisst	10,0	Exitus
"	64	240	"	2,5	mittel
"	65	260	"	5,0	stark
8/XII	172	160	L. H. F.	0,5	stark
"	173	160	"	1,0	Exitus
"	171	160	ditto. Essig, enteiweisst	0,5	"
"	174	190	"	1,0	"

Bei dem Autolyseversuche wird ein Teil von Askaridenleibesbrei, frei von Leibeshöhlenflüssigkeit, auf dem Wasserbade bei 50° C getrocknet und pulverisiert und die anderen wurden nach bestimmten Brutstunden gleicherweise behandelt. Unter ihnen aber ist keine Vermehrung der Toxizität zu bemerken. Wir haben ferner den Rückstand von mit Wasser wiederholt extrahiertem, entfettetem Pulver mit Pepsin oder Trypsin verdauen lassen. Die Askaronfraktion aus dem Verdauungsprodukte war weit weniger toxisch als das Rohaskaron.

TABELLE XXVII.

## Autolyseversuch.

Datum.	Nummer.	Gewicht g.	Brutstunde.	Dosis mg.	Resultat.
6/IX	105	265	0	2,5	stark
	108	330	0	5,0	schwach
	106	290	48	2,5	stark
	109	330	"	5,0	"
	110	295	96	2,5	schwach
	107	280	144	"	"
8/IX	113	225	0	10,0	Exitus
	114	230	0	5,0	schwach
	118	"	0	10,0	stark
	116	240	6	5,0	mittel
	115	249	12	"	schwach
	117	265	24	"	"
	119	215	24 (in Alkohol)	10,0	Exitus
	120	204	"	5,0	schwach

Wir können noch nicht entscheiden, ob gerinnbares Eiweiss von Askarien die Muttersubstanz von Askaron sei oder nicht; die Beobachtung, dass Askaron hauptsächlich in der Leibeshöhlenflüssigkeit enthalten ist, dass verschiedene Gewebe eine gleiche Toxizität haben, und dass die Helfminthen, welche in den verschiedenen Organen wie Magendarmkanal, Bauchhöhle und Herz etc. parasitieren, ungeachtet ihrer Medien die gleiche giftige Substanz haben, dass sie das Gift oder ihre Muttersubstanz nicht aus den Medien hergenommen haben, zwingt uns anzunehmen, dass das Askaron nicht ein zweckdienliches Sekret sondern ein zufällig giftiges Stoffwechselprodukt von anoxybiotischen Helfminthen sei.

So ist die Frage nach der Identität des anaphylaktischen Shockes und der Askaronvergiftung noch offen. Wir können aber nur vermuten, dass das Askaron eine Art von Anaphylatoxin sei. Wenn man anzunehmen wagt, dass es verschiedene Anaphylatoxine gebe, so sollen diese bei der Vergiftung eine zellulär spezifische Resistenz und eine Gemeinresistenz zurücklassen und der Mechanismus von Antianaphylaxie aus drei Faktoren, d.i. einer spezifischen Resistenz (im Sinne FRIEDBERGERS), einer zellulär spezifischen Resistenz

und einer Gemeinresistenz bestehen. Dass das Serum von Tieren im antianaphylaktischen Stadium das normale passiv präparieren kann, soll allerdings einer lange dauernden zellulär spezifischen Resistenz und gleichzeitiger Anwesenheit von Antikörpern zugeschrieben werden.

## V. Immunisierungsversuche.

### 1. AKTIVE IMMUNISIERUNG.

Nach der aktiven Immunisierung ist die Entstehung von humoraler Immunität zu untersuchen, obgleich das schnelle Auftreten der Askaronresistenz die allerdings als fraglich voraussehen lässt. Wir haben zwei Pferde und ein Kaninchen im Intervalle von ca. 7 Tagen mit zunehmenden Dosen Askaron intravenös behandelt. Die Pferde wurden nach 19 maliger Injektion einer ca. 400 fachen Menge der letalen Dosis, und das Kaninchen nach 8 maliger Injektion dem 20 fachen der letalen Dosis resistent.

TABELLE XXVIII.

Aktive Immunisierung von Pferden.

A). Versuchspferd: Yamasakura.

Datum.	Gewicht kg.	Temperatur.	Rohaskaron mg.	Reaktion.
24/XI '15	429,6	37,4	0,05	schwach
"	"	—	"	"
27	408,5	37,9	0,2	"
2/XII	410,4	"	0,5	"
9	"	"	1,0	"
19	408,5	37,4	2,0	"
23	414,2	37,5	5,0	"
4/I '16	423,7	38,0	"	"
15	423,3	37,7	7,5	"
21	423,7	38,0	15,0	stark
27	423,0	37,5	30,0	negativ
1/II	423,7	"	60,0	stark
7	423,0	37,1	120,0	schwach
23	415,4	37,3	50,0	sehr schwach

Datum.	Gewicht.	Temperatur.	Rohaskaron mg.	Reaktion.
29	416,2	37,3	100,0	schwach
7/III	412,3	37,5	200,0	"
14	412,3	37,3	300,0	mittel
21	399,0	37,4	333,0	schwach
28	402,8	38,0	400,0	mittel

## B). Versuchspferd : Kirifu.

Datum.	Gewicht.	Temperatur.	Rohaskaron mg.	Reaktion.
15/XI '15	330,6	37,4	0,05 (c)	negativ
23	326,8	37,0	0,2	schwach
27	324,9	37,3	"	negativ
2/XII	323,0	37,5	5,0	sehr schwach
9	325,7	"	10,0	negativ
16	324,9	"	30,0	schwach
23	323,0	"	50,0	negativ
4/I '16	339,3	37,6	100,0	schwach
15	340,1	37,5	5,0 (v)	negativ
21	339,7	37,7	10,0	"
27	340,5	37,4	30,0	mittel
1/II	339,7	37,2	60,0	negativ
7	339,3	37,0	120,0	mittel
23	340,5	37,2	50,0	schwach
29	347,9	37,4	100,0	"
7/III	351,7	37,2	200,0	stark
14	372,4	37,5	300,0	"
21	351,4	37,1	500,0	Exitus

TABELLE XXIX.

Aktive Immunisierung von Kaninchen.

Kaninchen Nr. 12.

Datum.	Gewicht g.	Rohaskaron mg.	Bemerkung.
21/I '16	2175	2,0	negativ
22	2150	-	-

Datum.	Gewicht g.	Rohaskaron mg.	Bemerkung.
29	2180	—	negativ
25	2190	5,0	schwach
26	2285	—	—
27	2250	—	—
28	2240	—	—
29	2215	—	—
31	2272	—	—
1/II	2250	—	—
2	2280	—	—
3	2252	—	—
4	"	7,5	schwach
5	2230	—	—
7	2184	—	—
8	2102	—	—
9	2247	—	—
10	2222	—	—
11	2190	—	—
12	2150	—	—
14	2207	—	—
15	2235	10,0	negativ
16	2210	—	—
17	2240	—	—
18	2295	—	—
19	2310	—	—
20	2282	—	—
21	2250	—	—
22	2169	—	—
23	2170	—	—
24	2155	—	—
25	2147	—	—
26	2137	—	—
28	"	—	—
29	2177	30,0	negativ
2/III	2162	—	—
3	2165	—	—
4	"	—	—
6	2204	—	—
7	2240	50,0	mittel

Datum.	Gewicht g.	Rohaskaron mg.	Bemerkung.
8	2250	—	—
9	2295	—	—
10	2215	—	—
11	2290	—	—
13	2272	—	—
14	2248	—	—
16	2180	—	—
17	2155	—	—
18	2270	—	—
20	2240	—	—
21	2215	100,0	schwach
23	3305	—	—
24	2310	—	—
25	2332	—	—
27	2299	—	—
28	2287	—	—
29	2246	—	—
30	2255	—	—
31	2270	—	Versuch unterbrochen

Bei diesen Versuchen machen wir besonders darauf aufmerksam, dass ausser dem Reaktionsstadium, im Allgemeinbefinden, in der Körpertemperatur und im Gewicht die Versuchstiere keine nennenswerten Störungen konstatieren lassen. Und bei den zahlreichen Injektionsfällen wurde der Ernährungszustand von Meerschweinchen auch nicht gestört. Wie in der Einleitung erwähnt wurde, wollte SEYDERHELM die bei intravenöser Injektion schwere Störungen hervorrufende Substanz von *Gastrophiluslarven* auch als die Ursache von *Anaemia perniciosa infectiosa* bei Pferden betrachten. Mit dem Askaron, welches dieselben Störungen verursachen kann, konnten wir aber das Auftreten der perniziösen Anämie noch nicht konstatieren.

## 2. PASSIVE IMMUNISIERUNG.

Wir haben mit den Immunseris aus den Pferden Yamasakura und Kirifu nach den folgenden drei Schemata Meerschweinchen passiv zu immunisieren

versucht. Wie aus den Tabellen XXX a, b, c, ersichtlich ist, eine Schützwirkung von „Immunserum“ nicht bemerkbar.

a). Rohaskaron wird in Immunserum gelöst und nach 1 stündigem Stehen bei 38° C im Brutschrank Meerschweinchen intravenös injiziert.

b). Meerschweinchen werden mit Immunserum intraperitoneal behandelt und ihnen nach einem 24 stündigen Intervalle Rohaskaron intravenös injiziert.

c). Ein Gemisch in Verhältnis von 0,7 ccm Immunserum, 0,8 mg Rohaskaron und 1,3 ccm Meerschweinchenserum (Komplement) wird bei 38° C eine Stunde lang im Brutschrank gestellt und dann intravenös einverleibt.

### TABELLE XXX.

#### Passive Immunisierung von Meerschweinchen.

a, N.S. normales Pferdeserum. K.S. Immunserum aus Kirifu (120 fache L.D. resistent.) Y.S. Immunserum aus Yamasakura (120 fache L.D. resistent.) Immunsera wurden am 14. Tage nach der letzten Injektion entnommen.

Datum.	Nummer.	Gewicht g.	Serum + Rohaskaron. ccm.	mg.	Resultat.
22/II '16	187	240	—	0,2	Exitus
	188	360	—	„	schwach
	190	260	—	„	Exitus
	191	250	K.S. 1,9	—	negativ
	193	235	N.S. 0,5	0,2	mittel
	186	240	Y.S. „	..	Exitus
	192	267	„	..	schwach
	194	220	„	0,4	stark
	187	230	K.S. „	0,2	Exitus
	197	295	Y.S. „	0,6	„

b, K.S. Serum aus Kirifu (120 fache L.D. resistent.) Y.S. (II. 22-23) Serum aus Yamasakura (120 fache L.D. resistent.) N.S. normales Serum. Y.S. (IV. 7-8) Serum aus Yamasakura (400 fache L.D. resistent.) Immunpferde wurden am 14. Tage nach der letzten Injektion entblutet.

Datum.	Nummer.	Gewicht g.	Serum ccm.	Rohaskaron mg.	Resultat.
22-23/II '16	201	350	—	0,5	Exitus
	195	220	Y.S. 5,0	„	schwach

Datum.	Nummer.	Gewicht g.	Serum ccm.	Rohaskaron mg.	Resultat.
22-23/II '16	198	190	Y.S. 5,0	0,5	Exitus
	199	235	K.S. "	"	"
	200	201	"	"	negativ
7-8/IV	296	155	N.S. "	0,4	Exitus
"	297	185	10	0,6	"
"	298	180	"	0,4	"
"	303	180	Y.S. 5	"	stark
"	304	185	"	"	Exitus
"	299	200	10	"	sehr stark
"	300	200	"	"	Exitus
"	301	195	"	"	stark

c, Serum aus Yamasakura (400 fache L.D. resistent) am 14. Tage nach der letzten Injektion genommen. ( ) Kontrolltier.

Datum.	Nummer.	Gewicht g.	Rohaskaron mg.	Resultat.
9/IV '16	(305)	245	0,4	mittel
"	306	255	"	Exitus
"	(307)	250	"	"
"	308	245	"	"
"	309	250	"	"
"	311	220	0,2	"
"	(312)	215	"	stark

### 3. KOMPLEMENTABLENKUNG UND PRÄZIPITINREAKTION.

Die Komplementablenkung und die Präzipitinreaktion sind, wie schon erwähnt wurde, bei normalem Pferdeserum immer negativ ausgefallen. Beim Immunserum gegen das Askaron werden die Resultate dieser Versuche nicht nur mit dem Askaron sondern auch mit der Askaronfraktion aus den Pferdeaskariden positiv bestätigt. Da das Rohaskaron mit Spuren von Lipoiden und Glykogen verunreinigt sein konnte, so haben wir zur Kontrolle einen alkoholischen Askaridenextrakt und reines Glykogen und auch Peptonum Witte als Antigen benutzt ohne positive Resultate zu bekommen.

Die Präzipitinreaktion ist sehr schwach, aber ein Unterschied mit der

Kontrolle wird deutlich konstatiert. (Die Versuchsschemata sind gleich denen bei normalen Seris.)

TABELLE XXXI.\*

Komplementablenkung mit Immunpferdeserum und Rohaskaron.

Datum.	Namen.	Serum ccm.	Antigen mg.	Titer v. Zieg. Kaninch- serum.	Resultat.	Bemerkung.
9/XI'15	Shuntei	0,5	Rohaskaron 0,4-0,00001	1:500	+A. 0,025	I. 3.
18/XII	Yamasakura	"	0,4-0,008	1:1000	+A. 0,0125	I. 6 R. 2.
"	Kirifu	"	"	"	+A. 0,025	I. 6 R. 30mg subk.
14/I'16	Yamasakura	"	0,4-0,0001	1:500	+A. 0,00625	I. 8 R. 5.
"	Kirifu	"	"	"	+A. 0,0125	I. 8 R. 100mg subk.
21/II	Yamasakura	0,3	"	"	+A. "	I. 13 R. 120.
"	Kirifu	0,25	"	"	+A. "	"
"	Yamasakura	1-0,00006	0,4	"	+S. 0,25	"
"	Kirifu	"	"	"	+S. "	"
"	Yamasakura	1-0,03	witte Pepton 0,4	"	-	"
"	Kirifu	"	"	"	-	"
"	Yamasakura	"	Glykogen 0,4	"	-	"
5/IV	"	0,5-0,002	Rohaskaron 0,1	1:1500	+S. 0,0625	I. 18. R. 333.
"	"	0,5	0,4-0,0008	"	+A. 0,05	"
2/V	"	0,5-0,002	0,1	1:1000	+S. 0,125	I. 19. R. 400
"	"	"	alk. Ext. v. asc. lumb. 0,25	"	-	"
"	"	"	Rohask. aus asc. meg. 0,1	"	+S. 0,5	"

TABELLE XXXII.\*

Präzipitinreaktion.

Datum	Namen.	Serum. %	Antigen. %	Resultat.	Positives Minimum.	Bemerkung.
16/XI'15	Shuntei	100-6,25	Rohask. 0,1	±	S. 25,0	I. 4. R. 5,0mg (subk.)
22	"	"	"	"	S. 12,5	"

\* I=Injektionsmahl, A bezw. S bedeutet minimale Komplementablenkungsmenge von Rohaskaron bzw. Serum. R=Resistenzgrad.

Datum.	Namen.	Serum. %	Antigen. %	Resultat.	Positives Minimum.	Bemerkung.
22	Yuwane	100-6,25	Rohask. 0,1	±	S. 25,0	I. 6. R. 5,0 mg
23	"	"	"	"	"	"
27	Yamasakura	"	"	+	"	I. 2. R. 0,2 mg
"	Kirifu	"	"	"	"	I. 2. R. 0,2 mg
2/XII	Yamasakura	"	"	"	"	I. 3.
"	Kirifu	"	"	-	-	I. 3. R. 0,2 mg
9	Yamasakura	"	"	+	S. 12,5	I. 4. R. 0,5 mg (v)
"	Kirifu	"	"	"	"	I. 4. R. 5,0 mg (subk.)
11	Yamasakura	"	"	"	S. 25,0	I. 5.
"	Kirifu	"	"	-	-	I. 5. R. 10,0 mg
14/I'16	Yamasakura	"	"	+	S. 25,0	I. 8. R. 5. fache (v)
"	Kirifu	"	"	-	-	I. 8. R. 3,0 mg
5/IV	Yamasakura	100	1-0,065	+	A. 0,125	I. 18. R. 333 fache (v)

Wir haben bei Pferden nicht nur die Entstehung von aktiver Immunität durch wiederholte Askaron-Behandlung, sondern auch das Auftreten von komplementablenkenden Antikörpern und von Präzipitinen in dem Immunserum als ganz sicher gefunden. Da die Entstehung von humoraler Immunität aber nie stattfand und das Immunserum keine Schützwirkung gegen das Askaron hatte, so müssen wir die aktive Immunität der hohen, zellulär spezifischen Resistenz zuschreiben.

## VI. Zusammenfassung.

1. Wir haben aus der Leibeshöhlenflüssigkeit und dem getrockneten Pulver von Askariden (*Ascaris lumbricoides* aus Schweinen und Menschen, *Ascaris megalocephala*) hochtoxische Albumosen-Peptone isoliert und für diesen Stoff den Namen „Askaron“ vorgeschlagen. Das Askaron ruft alle Vergiftungssymptome hervor, welche bei Askariase und bei Injektionen von Leibeshöhlenflüssigkeit oder von wässrigem Extrakt von Leibessubstanz konstatiiert werden. Seine Verbreitung in anderen Helminthen ist ziemlich gross, und die Askaronfraktion mit denselben Wirkungen und Toxizität wurden in *Filaria immitis*, *Gastrophiluslarven*, *Sclerostomum vulgare*, *Oxyuris curvula* und *Trichocephalus depressiusculus* nachgewiesen.

2. Aetherischer Extrakt und alkoholischer Extrakt von Askaridenrohpulver sind in grossen Dosen atoxisch. Sie sind aber hämolytisch wirksam, während im Askaron diese Wirkung vermisst wird.

3. Von den Versuchstieren sind Pferde am empfindlichsten für das Askaron, und danach folgen in absteigender Reihe Meerschweinchen, Hunde und Kaninchen. Ratten und Mäuse sind refraktär. Die wichtigsten Symptome bei der Askaronvergiftung sind Atemstörung, Erweiterung der peripheren Blutgefäße, Vermehrung der Absonderungen (Schwitzen, Tränen, Nasenschleim etc.) und Ausscheidungen (Drang zur Kotentleerung), nervöse Störungen, Temperatur- und Blutdrucksenkung, und autoptische Befunde zeigen Lungenblähung (bei Meerschweinchen), Blutung und hämorrhagisches Exsudat aus dem Magendarmkanal, Endokard und in inneren Organen (besonders in der Lunge), und unvollkommene Blutgerinnung. Die letale Dosis von Rohaskaron pro Kg Körpergewicht bei intravenöser Injektion ist bei Pferden 0,004 mg, bei Meerschweinchen 0,8 mg; bei Hunden 2,0 mg und bei Kaninchen 5,0 mg.

4. Die schwere Augenreaktion, welche hauptsächlich flüchtigen Substanzen von Askariden zugeschrieben wird, fällt mit stark verdünnter Lösung Rohaskaron (bis 0,1%) bei Pferden zu 100% (über 30 Fälle) positiv aus. Die Reaktion wird bei wiederholter Beträufelung immer schwächer, aber sie verschwindet nie.

5. Nach der Askaronvergiftung entsteht bedeutend schnell eine hohe Resistenz und die Askaronfraktionen aus verschiedenen Schmarotzern schützen sich in gleichem Grade gegeneinander.

6. Die Symptome, die anatomische Veränderung und Resistenzentstehung der Askaronvergiftung sind denen von anaphylaktischem Shocke ähnlich.

7. Askaron ist primär toxisch und hat keine Eigenschaften von Anaphylaktogen. In Seris von normalen Pferden lässt sich mittels des aktivopassiven Anaphylaxieversuches, des Komplementablenkungsverfahrens und der Präzipitinreaktion das Vorhandensein von Antikörpern gegen Askaron nicht nachweisen.

8. Das Pferd kann mit Askaron gegen über das 400 fache der letalen Dosis aktiv immunisiert werden und dabei werden komplementablenkende Antikörper und Präzipitine in Immunserum nachgewiesen, aber die Entste-

hung von humoraler Immunität nicht. Die hohe aktive Immunität muss einer zellularen Resistenz zugeschrieben werden.

9. Obgleich wir nichts über die Matrix von Askaron wissen, so können wir doch aus den Tatsachen, dass das Askaron bei den in verschiedenen Medien wie Darminhalt, Blut und Bauchhöhlenflüssigkeit lebenden Würmern weit verbreitet ist, dass es in der Leibeshöhlenflüssigkeit am sichersten enthalten ist, und dass die Giftigkeit von Würmern aus verschiedenen Organen dieselbe ist, zu dem Schlusse kommen, dass das Askaron nicht als zweckdienliches Sekret sondern als ein zufällig giftiges Stoffwechselprodukt zu betrachten ist.

10. Der Gemeingrad von Anaphylatoxinresistenz und Askaronresistenz ist gegen die Askaronresistenz selbst sehr schwach. Die Askaronresistenz besteht aus einer schwachen aspezifischen Resistenz und einer starken zellular spezifischen Resistenz.

11. Wenn man das Askaron als eine Art Anaphylatoxin ansehen darf, so muss in der Serumantianaphylaxie außer der spezifischen Resistenz und der aspezifischen Resistenz nach FRIEDBERGER noch die Entstehung von einer zellular spezifischen Resistenz vermutet werden.

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All communications relating to this Journal should be addressed to the Director of the College of Agriculture.

# Über die Bedeutung des Salzes bei der Agglutination.

VON

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## Einleitung.

Seitdem BORDET (1) gezeigt hat, dass zum Eintritt der Agglutination eine kochsalzhaltige Flüssigkeit notwendig ist, und diese Beziehung in Analogie gesetzt hat zu der Tatsache, dass eine feinste Tonaufschwemmung in Wasser durch Kochsalz gefällt wird, und ferner Joos (2) BORDET's Versuch bestätigt hat, nach welchem salzfreie Bazillen und salzfreies agglutinierende Serum in sonst wirksamen Konzentrationen keine Reaktion erzeugen, ist die Notwendigkeit der Gegenwart von Kochsalz nicht mehr bezweifelt worden.

Es verbinden sich jedoch manchmal die Bakterien und die entsprechenden Agglutinine, wenn auch das Salz nicht gegenwärtig ist. Nach FRIEDBERGER (3) spielte nicht nur Kochsalz, sondern auch die Salze der Alkalien, alkalischen Erden, Halogene oder organischen Kristalle resp. Asparagin oder Traubenzucker bei der Agglutination eine Rolle.

Salz selbst mag in einer Konzentration Bakterien agglutinieren, und es liegt da die Schwierigkeit vor, eine echte Agglutination von Pseudoagglutination zu unterscheiden. EISENBERG und VOLK (4) fanden, dass Neutralsalze je nach der Konzentration bald einen fördernden, bald einen hemmenden Einfluss auf die Agglutination haben.

Bakterien werden durch Säuren und durch die Salze der Schwermetalle in geringer Konzentration gefällt, während Alkalien, alkalische Erden und Erdmetalle erst in ausserordentlich höher Konzentration wirksam sind.

Die Experimente mit Alum von TSUSHIMA, meinem Kollegen, lehren uns, dass Dysenterie-, Typhus- und Kolibazillen durch Alum in ziemlich

geringer Konzentration gefällt werden, aber ein Stamm der Kolibazillen gar nicht.

Es gibt mehrere Hypothesen vom Wesen der Wirkung des Salzes bei der Agglutination. JOOS unterscheidet am Vorgang zwei Phasen; erstens Vereinigung der mit Agglutinin beladenen Mikroben und des Salzes zu Flocken, was eine chemische Verbindung sei. Während ALTBELLI und MEMME (5) nur Vermutungen aufstellen, in welcher Weise die Mineralsubstanzen von Bedeutung für die Agglutination wären, durch Fällung der Proteine, oder Beeinflussung der Osmose oder Änderung der Molekularattraktion und Adhäsion, haben NEISSEL und FRIEDEMANN (6) und BECHHOLD (7) die Rolle der Salze für unorganisierte und für Bakterien-Suspensionen genauer studiert. Sie stellen die Schwellwerte fest, d.i. jene geringste Salzkonzentration, bei welcher nach 24 Stunden noch die Ausflockung erfolgt. Dabei steht der Schwellwert der Salze in einer gewissen Relation mit der Entladungsspannung der Metalle, d.i. die Kationen mit niedriger Entladungsspannung besitzen ein hohes, die mit hoher Entladungsspannung ein sehr geringes Fällungsvermögen, doch gibt es auch Unregelmäßigkeiten in der Reihe. Und es ergibt sich, dass kein prinzipieller Unterschied zwischen der Ausflockung von Bakterien-Agglutininen und Kolloiden besteht. Nach HARDY (8) eignet sich die Fällung der Kolloide zur Neutralisation der Elektrizität zwischen Dispersoiden und Ionen oder anderen Kolloiden, welche gegenseitige Elektrizität beladen, dabei erzeugt der entstandene Niederschlag keine Kataphorese. Allein FRIEDEMANN (9) fand, dass Bakterien durch salzfreies Serum agglutiniert werden, doch scheinen hierbei, wie er selbst annimmt, die Globuline eine Rolle zu spielen.

Nun beschreibe ich einige Experimente von FRIEDEMANN:

#### TABELLE 1.

Agglutinationsversuch mit normalem Kaninchenserum durch Typhusbazillen  
(nach FRIEDEMANN).

Serum.	Dünne Bakterienaufschw.	In reinem Wasser	3 Tropfen 10% NaCl-Lös.
1 : 2	1 Tropfen	+++	+++
1 : 4	"	+++	-
1 : 8	"	+++	-

Serum.	Dünne Bakterienaufschw.	In reinem Wasser	3 Tropfen 10% NaCl-Lös.
1 : 16	1 Tropfen	+++	—
1 : 32	"	+++	—
1 : 64	"	+++	—
1 : 128	"	++	—
1 : 256	"	++	—
1 : 512	"	+++	—
1 : 1024	"	+++	—
1 : 2048	"	—	—
1 : 4096	"	—	—

Beobachtung nach 2 Stunden bei 37°C., nach 20 Stunden bei Zimmertemperatur.

Normales Kaninchenserum wird 4 Tage gegen fliessendes Wasser dialysiert und filtriert.

Es zeigt sich, dass die Höhe, in der ein Serum in salzfreier Lösung agglutiniert, von seinem Titer in salzhaltiger Lösung unabhängig ist, und dass Normalsera sich in dieser Beziehung den Immunsera durchaus gleich verhalten. So gab auch ein normales Kaninchenserum noch in der Verdünnung 1:1000 in salzfreier Lösung deutliche Agglutination, während es in salzhaltiger Lösung fast gar nicht wirkte.

Beachtungswert sind die Experimente mit Immunsera nach FRIEDEMANN, wie Tabelle 2 zeigt.\*

TABELLE 2.

Agglutinationsversuch mit Immunkaninchenserum durch Typhusbazillen  
(nach FRIEDEMANN).

Serum.	Dünne Bakterienaufschw.	In reinem Wasser	3 Tropfen 10% NaCl-Lös.
1 : 2	1 Tropfen	+++	+++
1 : 4	"	+++	+++
1 : 8	"	+++	+++
1 : 16	"	+++	+++
1 : 32	"	+++	+++

Serum.	Dünne Bakterienaufschw.	In reinem Wasser	3 Tropfen 10% NaCl-Lö.
1 : 64	1 Tropfen	+++	+++
1 : 128	"	+++	+++
1 : 256	"	—	+++
1 : 512	"	+++	+++
1 : 1024	"	+++	+++
1 : 2048	"	—	+++
1 : 4096	"	—	+++

Beobachtung nach 2 Stunden bei 37°C., nach 20 Stunden bei Zimmer-temperaturen.

Immunkaninchenserum wird 4 Tage gegen fliessendes Wasser dialysiert und filtriert.

Will man nicht die Annahme machen, dass die Spezifität der Agglutinationsreaktion nur in salzhaltiger Lösung in die Erscheinung tritt, so spricht diese Feststellung wohl sehr dafür, dass die Substanzen, welche in salzfreier Lösung wirken, mit den spezifischen Agglutininen nicht identisch sind.

FRIEDEMANN beobachtete, wenn er mit einer Bakterienart (Typhus, Koli, Vibrio Metschnikoff) absorbierte, und nunmehr untersuchte, ob das Agglutinin für diese verschwindet, wie es sich für die anderen Bakterienarten verhielt. Auch bei diesen Versuchen stieg häufig das Agglutinationsvermögen erheblich, und zwar bisweilen nicht nur für die gleiche Art, sondern auch für eine der anderen. Bereits fand BORDET, dass bei Eintragung von Choleravibrionen, wenn solche nicht mehr agglutinierten, dies noch mit Typhusbazillen der Fall war. In analoger Weise haben auch EISENBERG und VOLK die Absorption des normalen Typhus-Agglutinins durch Typhusbazillen erwiesen.

In meiner Erforschung der Agglutination habe ich mehrmals gesehen, dass ein Typhus-Immunkaninchenserum bis zu 5000 facher Verdünnung in 0,85%iger NaCl-Lösung Typhusbazillen agglutiniert, während dieses Serum bis zu 100 facher Verdünnung in destilliertem Wasser dieselben Bazillen agglutinierte. Dabei erhielt ich aber nicht die Resultate, wie sie FRIEDEMANN in vorliegenden Tabellen zeigte. Ich habe einige zweifel über seine Beschreibung der Resultate der Experimente, in seinen Tabellen zeigte er hohen Agglutina-

tionstiter des spezifischen Agglutinins in salzfreier Lösung und in seiner Beschreibungstellte er auf, dass die spezifischen Agglutinine in salzfreien Lösungen unwirksam sind.

Es ist keine zweifelhafte Tatsache, dass Immunserum gegen entsprechenden Bakterien bei stärksten Verdünnungen agglutinieren mag [PFEIFFER und KOLLE (10), GRUBER und DURHAM (11)], je doch ist es zunächst unerichtlich, dass Rotzbazillen durch Normalsera der verschiedenen Arten bei stärksten Verdünnungen agglutiniert werden, wie Tabelle 3 zeigt.

TABELLE 3.

(nach FEDOROWSKY (12)).

Arten des Serums.	Makroskopisch.	Mikroskopisch.
Mensch.....	165—500	330—1000
Pferd.....	165—330	330—500
Kaninchen .....	165—336	250—600
Katze.....	200—250	330—650
Ochs .....	250	500—650
Hund.....	150—500	250—1000
Meerschweinchen .....	165—500	330—1000
Schaf .....	250—330	300—1000
Ziege .....	250—500	500—1000
Schwein.....	330—500	500—1000
Kuh .....	330—500	830—1000
Maus.....	330—630	330—1000
Vogel.....	650—1000	1000—1255

### Meine Versuche.

Absicht der Versuche: Ich habe mehrmalige Versuche bei Agglutination mit Normal- und Immunserum gemacht, um zu finden, ob das Salz eine wichtige Rolle spielt, und ich glaube entscheidende Resultate erhalten zu haben.

*A. Material der Versuche.*

1. Stämme der Bazillen.

Typhusbazillen: Eine 24 Stunden alte Kultur auf einem Schiefer-Agar von Typhusbazillen, die in diesem Institute aufbewahrt wurden, werden in 10 cem. 0.85%iger NaCl-Lösung oder destilliertes Wasser aufgeschwemmt.

Kolibazillen: Ebenso wie Typhusbazillen.

Rotzbazillen: Eine 48 Stunden alte Kultur auf einem schwach alkalischen Glyzerinagar von Rotzbazillen, die in der Schule für Militär-Tierärzte aufbewahrt wurden, werden in 5 cem. 0.85%iger NaCl-Lösung oder destillierten Wassers aufgeschwemmt.

2. Normalsera aus kleinen Tieren.

Ausser dem Pferdeserum wurden Meerschweinchen-, Kaninchen- und Hundeserum verwandt; alle diese Tiere haben diesem Institute gehört.

3. Normalpferdeserum.

Ein serum, von dem aus der Vena jugularis entnommenen Blut geschieden, wird verwandt. Diese Pferde haben diesem Institute und der Schule der Militär-Tierärzte gehört.

4. Typhus-Immunkaninchenserum.

Eine Menge von 1/10, 1/5, 1, und 2 Normalösen der auf 60°C. 30 Minuten lang erhitzten und abgetöteten 24-stündigen Typhusbazillen wurde in die Ohrvene des Kaninchens mit eine Woche langem Abstände intravenös eingespritzt, und eine Probeentblutung am 10. Tage nach der letzten Impfung ausgeführt, und dessen Agglutinationstiter, über 20000 fach gegen entsprechendes Agglutinogen agglutinierend, wird bestimmt: am nächsten Tage wird totale Blutentnahme aus Arteria Carotis ausgeführt und so erhaltenes Serum in der Eiskammer aufbewahrt.

5. Rotz-Immunhundeserum.

Eine Menge von 1/10, 1/5, und 1 Normalöse der auf 60°C. 30 Minuten lang erhitzten und abgetöteten 24-stündigen Rotzkultur wird in die Jugularvene des Hundes, diesem Institute gehörend, mit einer Woche langen Abständen intravenös eingespritzt, eine Blutentnahme von einer brauchbaren Menge aus derselben Vene am 7. Tage nach der letzten Impfung ausgeführt, so erhaltenes Serum in der Eiskammer aufbewahrt.

### 6. Rotz-Immunpferdeserum.

Eine Menge von 1 und 2 normalen Ösen des oben beschriebenen Materials wird in die Jugularvene mit einer Woche langem Abstande einer Reihe von Pferden, und eine Menge von 1 Normalöse desselben Materials einer anderen Reihe von Pferden eingespritzt; alle diese Pferde haben der Schule der Militär-Tierärzte gehört.

### 7. Die Zeitdauer des Agglutinationsversuches.

Beobachtung nach 2 Stunden bei 37°C., danach 22 Stunden bei Zimmertemperatur für Typhusagglutination; Beobachtung nach 24 Stunden bei 37°C. für Rotzagglutination.

*B.* Verhalten zwischen Agglutinationstiter und An- und Abwesenheit des Salzes durch Typhus- und Rotzbazillen mit Normalpferde- und Normalhundeserum.

Versuchsmethode: Normalserum wird stufenweise mit destilliertem Wasser oder 0,85%iger NaCl-Lösung verdünnt, und alle Reagenzgläser werden auf 1 ccm. aufgefüllt, danach werden 2 Tropfen der Bazillenaufschwemmungen in alle Reagenzgläsern eingetropft und durch Schütteln gemischt. Die Art der Beobachtungen wurde schon oben beschrieben.

Alle folgenden Versuche werden mit derselben Methode ausgeführt wenn keine spezielle Beschreibung beim Versuche angezeigt wird.

Die Resultate dieser Versuche zeigen sich in folgenden Tabellen.

TABELLE 4.

Agglutinationsversuch durch Typhus- und Rotzbazillen mit Normalhundeserum  
(besonders mit Bezug auf An- oder Abwesenheit des Salzes).

#### 1. Durch Typhusbazillen

Verdünnung des Serums.	10	15	20	30	40	60	Kontrolle.
Aqua dest.....	+	+	+	±	±	—	—
0,85% NaCl-Lös ...	±	±	—	—	—	—	—

## 2. Durch Rotbazillen.

Verdünnung des Serums.	10	15	20	30	40	60	Kontrolle.
Aqua dest.....	+	+	+	±	±	—	—
0,85% NaCl-Lös....	±	±	—	—	—	—	—

TABELLE 5.

Agglutinationsversuch durch Rotbazillen mit Normalpferdeserum  
(besonders mit Bezug auf An- oder Abwesenheit des Salzes).

Verdünnung des Serums.	10	20	30	40	60	80	120	Kontrolle.
No. 1.								
16 Jahre alt { 0,85% NaCl-Lös. (Kastriert.) { Aqua dest .....	+	--	—	—	—	—	—	—
	+	+	—	—	—	—	—	—
No. 2.								
10 Jahre alt { 0,85% NaCl-Lös. (Kastriert.) { Aqua dest .....	+	±	—	—	—	—	—	—
	+	+	+	±	—	—	—	—
No. 3.								
7 Jahre alt { 0,85% NaCl-Lös. ( ♂ ) { Aqua dest .....	±	±	—	—	—	—	—	—
	+	+	+	±	—	—	—	—
No. 4.								
7 Jahre alt { 0,85% NaCl-Lös. ( ♀ ) { Aqua dest .....	+	+	+	±	—	—	—	—
	+	+	+	+	+	±	—	—
No. 5.								
10 Jahre alt { 0,85% NaCl-Lös. (Kastriert.) { Aqua dest .....	+	+	+	+	±	—	—	—
	+	+	+	+	+	±	—	—

Bemerkungen:

1. KOLLE und OTTO (13) betonen, dass das aus Normalagglutinin abgestammte Agglutinat durch die Schüttelung die Emulsion verändern möchte, und ich habe daher den Agglutinationstiter, damit die Reaktion durch die

Schüttelung nicht verschwinden möchte, als positiv angenommen. Diese Bemerkung gilt auch für die folgenden Versuche, bei Beurteilung der Reaktionen.

2. Die Grenzen der Ausflockung durch die Fällung des Globulins selbst mit destilliertem Wasser sind immer niedriger als der Normalagglutinationstiter.

Beurteilung der Resultate: Die Agglutinationsreaktion mit Normalserum verhält sich deutlicher in der salzfreien Lösung, und in diesem Falle scheinen auch Alter und Geschlecht des Tieres keine wichtige Rolle mit Bezug auf dem Titer zu spielen.

B. Versuch der Agglutination mit Normalhunde- und Normalpferdeserum, durch NaCl-Lösungen in verschiedenen Konzentrationen. Die Resultate dieser Versuche zeigen sich in folgenden Tabellen.

TABELLE 6.

Agglutinationsversuch mit dem in verschieden konzentrierten NaCl-Lösungen verdünnten Normalpferdeserum durch Rotbazillen.

Verdünnung des Serums.	5	10	15	20	40	60	80	120	160	320	Kontr.
% der NaCl-Lös. zur Verdünnung des Serums.											.
0 % .....	++	++	++	++	+	—	—	—	—	—	—
0,01 „ .....	++	++	++	++	+	+	±	—	—	—	—
0,02 „ .....	++	++	++	++	++	++	+	+	+	—	—
0,03 „ .....	++	++	++	++	++	++	++	+	+	±	—
0,1 „ .....	++	++	++	++	+	+	+	—	—	—	—
0,2 .....	++	++	++	+	±	±	±	—	—	—	—
0,3 .....	++	++	+	+	±	±	±	—	—	—	—
0,4 .....	++	++	+	+	±	±	±	—	—	—	—
0,5 .....	++	++	+	+	±	±	±	—	—	—	—
0,6 .....	++	+	+	+	±	±	±	—	—	—	—
0,7 .....	+	+	+	+	±	—	—	—	—	—	—
0,85 .....	+	+	+	±	—	—	—	—	—	—	—

TABELLE 7.

Verdünnung des Serum.	200	300	400	800	1200	2400	3200	4800	6400	9600	12800	25600	38400	Kontr.
0,04 % .....	++	++	++	++	++	+	+	-	-	-	-	-	-	-
0,05 „ .....	++	++	++	++	++	++	++	+	+	+	+	+	+	-
0,06 „ .....	++	++	++	++	++	++	++	+	+	+	+	+	+	-
0,07 „ .....	++	++	++	++	+	+	+	-	-	-	-	-	-	-
0,08 „ .....	++	++	++	+	+	+	+	-	-	-	-	-	-	-
0,09 „ .....	++	++	++	+	+	+	+	-	-	-	-	-	-	-

TABELLE 8.

Agglutinationsversuch mit dem in verschiedenen konzentrierten NaCl-Lösungen verdünnten Normalhundeserum durch Rotbazillen.

Verdünnung des Serums.	50	100	200	400	800	1600	3200	6400	12800	25600	Kontr.
% der NaCl-Lös. zur Verdünnung des Serums.											
0,85 % .....	-	-	-	-	-	-	-	-	-	-	-
0,7 „ .....	+	-	-	-	-	-	-	-	-	-	-
0,5 „ .....	+	+	-	-	-	-	-	-	-	-	-
0,3 „ .....	+	+	+	+	-	-	-	-	-	-	-
0,1 „ .....	+	+	+	+	+	-	-	-	-	-	-
0,08 „ .....	+	+	+	+	+	+	-	-	-	-	-
0,06 „ .....	+	+	+	+	+	+	+	-	-	-	-
0,05 „ .....	+	+	+	+	+	+	+	+	-	-	-
0,03 „ .....	+	±	-	-	-	-	-	-	-	-	-
0,01 „ .....	+	-	-	-	-	-	-	-	-	-	-
Aqua dest.....	±	-	-	-	-	-	-	-	-	-	-

In diesem Falle wurden die Kontrollversuche mit besonderer Vorsicht angestellt, weil die Bazillen (besonders Rotbazillen) beim Eintritt der Normalagglutination, wenn da eine passende Menge des Salzes vorhanden ist, spontan agglutinierbar sind, und um die Anwesenheit unreiner Substanzen oder Irrtum

in der Zubereitung der Bakterienaufschwemmung zu vermeiden. Dieser Versuch wurde mit durchaus chemisch reinen Reagenzgläsern mehrmals wiederholt. Beurteilung der Resultate: Bei der Anwesenheit nur einer Spur von NaCl erscheint der Eintritt der Normalagglutination deutlicher positiv als bei der absoluten Salzfreiheit. Wenn man das Normalserum mit der NaCl-Lösung in der passenden Konzentration verdünnt und Bazillen mit dieser Lösung aufschwemmt, kann man keinen Unterschied zwischen Normalagglutination mit jener Lösung und Immunagglutination mit 0,85%iger NaCl-Lösung finden.

C. Agglutinationsversuche mit Typhus-Immunkaninchenserum und Rotz-Immunkonjugat, mit NaCl-Lösungen in verschiedenen Konzentrationen verdünnt. Die Resultate dieser Versuche zeigen folgende Tabellen.

TABELLE 9.

Agglutinationsversuch mit dem in verschiedenen konzentrierten NaCl-Lösungen verdünnten Typhus-Immunkaninchenserum durch Typhusbazillen.

TABELLE 10.

Agglutinationsversuch mit dem in verschiedenen konzentrierten NaCl-Lösungen verdünnten Rotzimmunpferdeserums durch Rotbazillen.

Verdünnung des Serums.	10	20	40	80	160	320	640	1280	2560	5120	10240	20480	40960	Kontr.
% der NaCl-Lös. zur Verdünnung des Serums.														
Aqua dest.....	++	++	+	-	-	-	-	-	-	-	-	-	-	-
0,10 % .....	++	++	+	++	-	-	-	-	-	-	-	-	-	-
0,02 , .....	++	++	+	++	-	-	-	-	-	-	-	-	-	-
0,03 , .....	++	++	+	+	++	-	-	-	-	-	-	-	-	-
0,04 , .....	++	++	++	+	+	++	-	-	-	-	-	-	-	-
0,05 , .....	++	++	++	++	+	++	++	-	-	-	-	-	-	-
0,06 , .....	++	++	++	++	++	++	+	+	-	-	-	-	-	-
0,07 , .....	++	++	++	++	++	++	+	+	+	-	-	-	-	-
0,08 , .....	++	++	++	++	++	++	++	+	+	-	-	-	-	-
0,09 , .....	++	++	++	++	++	++	++	++	+	+	-	-	-	-
0,1 , .....	++	++	++	++	++	++	++	++	++	+	+	+	+	-
0,2 , .....	++	++	++	++	++	++	++	++	++	+	+	+	+	-
0,3 , .....	++	++	++	++	++	++	++	++	++	+	+	+	+	-
0,4 , .....	++	++	++	++	++	++	++	++	++	+	+	+	+	-
0,5 , .....	++	++	++	++	++	++	++	++	++	+	+	+	+	-
0,6 , .....	++	++	++	++	++	++	++	++	++	+	-	-	-	-
0,7 , .....	++	++	++	++	++	++	++	++	++	+	-	-	-	-
0,85 , .....	++	++	++	++	++	++	++	++	++	+	-	-	-	-

(Versuchspferde „Yakei“ und „Kanryo“ wurden am 11/I. mit je 1 Normal-Öse von auf 60°C. 30 Minuten lang erhitzten Rotbazillen intravenös eingespritzt.)

Beurteilung der Resultate: Zum Eintritt der Agglutination mit dem Immunserum gegen das Normalserum scheint sowohl 0,85% als auch solche Konzentration des Salzes (nicht 0,85%) zu geeignet sein, bei welcher der Immunagglutinationstiter sich am höchsten zeigt, wenn man Bazillen und Serum mit der NaCl-Lösung in jener Konzentration behandelt hat.

D. Versuch der Agglutination mit Menschen- und Meerschweinchen-serum.

Die Resultate macht Tabelle 11 ersichtlich.

TABELLE 11.

Agglutinationsversuch mit Normalmensch-, Normalkaninchen- und Normalmeerschweinchenserum.

Durch Rotzbazillen.

Verdünnung des Serums.	10	20	30	40	60	80	160	Kontr.
Kaninchenserum ...	Aqua dest .....	+	+	+	+	+	+	±
	0,8 % NaCl-Lös.	+	+	±	—	—	—	—
Meerschweinchenserum ...	Aqua dest .....	+	+	+	+	+	+	±
	0,85 % NaCl-Lös.	+	±	—	—	—	—	—

Durch Typhusbazillen.

Verdünnung des Serums.	10	20	30	40	60	80	160	Kontr.
Kaninchenserum ...	Aqua dest .....	+	±	—	—	—	—	—
	0,85 % NaCl-Lös.	+	—	—	—	—	—	—
Menschen-serum ...	Aqua dest .....	+	+	±	—	—	—	—
	0,85 % NaCl-Lös.	+	±	—	—	—	—	—

Bemerkung: Zu diesem Versuche wurde der Rest des Menschenserums, welches bei Wassermann's Reaktion verwandt wurde, benutzt, d.i. Inaktivierung 30 Minuten lang auf 56°C.

Beurteilung der Resultate: Zum Eintritt der Normalagglutination nehmen die Reaktionsleistungen beim Mangel des Salzes deutlich zu, ebenso wie der oben beschriebene Versuch zeigt.

E. Versuch der Agglutination, welcher den Einfluss der An-oder Abwesenheit des Salzes zeigt, mit Rotz-Immunhundeserum und Rotz-Immumpferdeserum. Die Resultate macht Tabelle 12 ersichtlich.

TABELLE 12.

Agglutinationsversuch mit Rotzimmumpferdeserum und Rotzimmunhundeserum durch Rotz- und Kolibazillen, bei An- oder Abwesenheit von Salz.

Rotzimmumpferdeserum.

Serum	Bak.-Aufschw.	5	10	20	40	80	100	200	300	400	800	1200	2400	4800	Kontr.
No. 1	Rotzbaz. { Aqua dest 0,85% NaCl-Lös.	+	+	+	+	+	-	-	+	-	-	+	-	-	-
	Kolibaz. { Aqua dest 0,85% NaCl-Lös.	+	+	+	±	±	-	-	-	-	-	-	-	-	-
No. 2	Rotzbaz. { Aqua dest 0,85% NaCl-Lös.	+	+	+	+	+	-	-	-	-	-	-	-	-	-
	Kolibaz. { Aqua dest 0,85% NaCl-Lös.	+	+	+	+	+	+	+	+	+	+	+	+	±	-

(Versuchspferde No. 1 und No. 2 wurden am 11. und 14. April 1916 mit 1 und 2 Normalöszen der auf 60°C. 30 Minuten lang erhitzten Rotzbazillen intravenös eingespritzt. Am 20. April Probeaderlass.)

Rotzimmunhundeserum (durch Rotzbazillen).

Serum	Verdünnungsflüssigkeit	10	20	40	80	100	200	400	600	800	1000	1600	2400	4800	Kontr.
No. 1	{ Aqua dest .....	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	{ 0,85% NaCl-Lös. ..	+	+	+	+	+	+	+	+	+	+	+	+	-	-
No. 2	{ Aqua dest .....	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	{ 0,85% NaCl-Lös. ..	+	+	+	+	+	+	+	+	+	+	+	+	-	-
No. 3	{ Aqua dest .....	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	{ 0,85% NaCl-Lös. ..	+	+	+	+	+	+	+	+	+	+	+	+	-	-

(Versuchshunde No. 1, No. 2 und No. 3 wurden am 3, 8 und 13/III. 1916 mit 1/10, 1/5 und 1 Normalöse der auf 60°C. 30 Minuten lang erhitzten Rotzbazillen intravenös eingespritzt. Am 20/III Probeaderlass.)

Beurteilung der Resultate: Rotzimmunagglutinationstiter nimmt einen Grad bei reichlichem Vorhandensein des Salzes zu, wenn man Bazillen und Serum mit 0,85%iger NaCl-Lösung behandelt, ebenso wie Immunagglutinationsreaktion mit Typhusimmunserum zeigt.

Wenn man die Agglutinationsversuche mit Immunserum durch von Agglutinogen unberührte Bazillen nicht macht, dann wirkt dieses Immunserum auf diese Bazillen als Normalserum ein und sein Titer ist bei Mangel des Salzes höher.

F. Absorptionsversuch des Normalagglutinins im Normalserum. Ich habe schon in der Einleitung beschrieben, dass nach Friedemann bei Eintragung eines Stamms von Bazillen in Normalserum die nach Zentrifugierung abgehobene Flüssigkeit gegen die entsprechenden Bazillen durch ihre Agglutinabilität hervorragte, während nach Bordet bei Eintragung von Choleravibrionen in ein Choleravibrionen und Typhusbazillen agglutinierendes Normalserum die abgehobene Flüssigkeit Choleravibrionen nicht mehr agglutiniert, wohl aber noch Typhusbazillen.

Mein Absorptionsversuch zeigt jedoch, dass der Eintritt der Agglutinationsreaktionen mit Normalserum gegen andere Bazillen und entsprechende Bazillen nach dem Absorptionsvorgange ausserordentlich gehemmt wird, wie Tabelle 13 zeigt.

TABELLE 13.

Absorptionsversuch mit Normalagglutinin.

	100	200	300	400	800	1200	1600	2400	6400	9600	Konst.
Kolibaz. (I Kultur)	Rotzbaz.-Aufschw. (2 Tropfen)	±	—	—	—	—	—	—	—	—	—
—	wie oben	+	+	+	+	+	+	+	+	+	—
2 Stunden bei 37°C. 2 malige zentrif. in 30 Minuten.	Rotzbaz. (I Kultur)	Kolibaz.-Aufschw. (2 Tropfen)	±	—	—	—	—	—	—	—	—
—	wie oben	+	+	+	±	—	—	—	—	—	—
Rotzbaz. (I Kultur)	Rotzbaz.-Aufschw. (2 Tropfen)	±	—	—	—	—	—	—	—	—	—
Kolibaz. (I Kultur)	Kolibaz.-Aufschw. (2 Tropfen)	±	—	—	—	—	—	—	—	—	—

Absorptionsvorgang

Agglutination

(Normalserum wird mit 0,05%iger NaCl-Lösung verdünnt.)



Beurteilung der Resultate: Bazillen werden mit Kaninchenhämoglobinlösung agglutiniert.

B. Agglutinationsversuch durch Rotz- und Typhusbazillen mit Eiweiss.

Versuchsmethode: Ovalbumin wird mit destilliertem Wasser gelöst und Ovoglobulin mit Ovalbumin mittels 0,85%iger NaCl-Lösung, und jene in sich 7%iges Eiweiss enthaltende Flüssigkeit wird zu diesem Versuch als Stammlösung verwandt. Resultat dieses Versuchs macht Tabelle 15 ersichtlich.

TABELLE 15.

Agglutinationsversuch mit Eiweiss.

Art der Baz.	Art der Eiw.	Verdünnung aus erwähnten Lös.	1	2	4	8	16	32	64	128	3 6	Kontr.
Rotzbaz.	Ovalb.	Aqua dest.....	+	+	+	+	+	+	+	+	+	—
	Ovalb. u. Ovoglobul.	0,85%ige NaCl-Lös.	—	—	—	—	—	—	—	—	—	—
Typhusbaz.	Ovalb.	0,85%ige NaCl-Lös.	—	—	—	—	—	—	—	—	—	—
	Ovalb. u. Ovoglobul.	Aqua dest.....	+	+	+	+	+	—	—	—	—	—
		0,85%ige NaCl-Lös.	+	+	—	—	—	—	—	—	—	—
		0,85%ige NaCl-Lös.	+	+	—	—	—	—	—	—	—	—

Resultat des Versuchs: Bazillen werden mit Eiweiss agglutiniert.

C. Agglutinationsversuch mit verschiedenen Peptonen.

Versuchsmethode: Witte Pepton, Gehe-Pepton und Chapoteaut-Pepton werden einzeln mit destilliertem Wasser verdünnt, und so erhaltene Flüssigkeiten werden als 7%iges Pepton, ebenso wie der Prozentsatz des Eiweißes im Serum, zu diesem Versuche verwandt. Resultat des Versuchs ist aus Tabelle 16 ersichtlich.

TABELLE 16.

Agglutinationsversuch mit verschiedenen Peptonen.

Baz.	Art der Pep.	Verdünnung aus erwähnten Lös.	10	20	40	80	120	320	Kontr.
Rotzbaz.	Witte-Pep.	Aqua dest .....	+	+	+	+	+	+	+
		0,85%ige NaCl-Lös.	H	—	—	—	—	—	—
	Gehe-Pep.	Aqua dest .....	+	+	+	+	+	+	+
		0,85%ige NaCl-Lös.	H	—	—	—	—	—	—
	Chapoteut-Pep.	Aqua dest .....	+	+	+	+	+	+	+
		0,85%ige NaCl-Lös.	H	—	—	—	—	—	—
Typhusbaz.	Witte-Pep.	Aqua dest .....	+	+	+	+	+	+	—
		0,85%ige NaCl-Lös.	H	—	—	—	—	—	—
	Gehe-Pep.	Aqua dest .....	+	+	+	+	+	+	—
		0,85%ige NaCl-Lös.	H	—	—	—	—	—	—
	Chapoteut-Pep.	Aqua dest .....	+	+	+	+	+	+	—
		0,85%ige NaCl-Lös.	H	+	—	—	—	—	—

Resultat des Versuchs: Bazillen werden mit verschiedenen Peptonen agglutiniert.

#### D. Agglutinationsversuch mit Extrakt aus einigen Arten Fischfleisches.

Versuchsmethode: Verschiedene Arten Fischfleisches werden im Mörser zermalmt, danach mit Gaze gepresst, und dann die erhaltenen Flüssigkeiten mit 0,85%iger NaCl-Lösung verdünnt, sodass der Gehalt an Eiweiss ca. 7% beträgt, um dem Prozentsatz des Eiweißes im Serum gleich zu machen. Nun wird sie mit N/10 NaOH-Lös. neutralisiert und schliesslich zu diesem Versuche als Stammlösung verwandt. Die Prozente des Eiweißes in verschiedenen Arten des Fischfleisches sind wie folgt (nach NUKATA).

Arten des Fischfleisches.	Eiweissgehalt (%)
Meerbrassenfleisch .....	18,97
Schollenfleisch .....	19,16

Karpfenfleisch .....	17,99
Karauschenfleisch .....	17,70

Die Resultate dieses Versuchs zeigt folgende Tabelle.

TABELLE 17.

Agglutinationsversuch mit Extrakt des Fischfleisches  
Durch Kolibazillen.

Fleisch	Lösung zur Extrahierung.	Verdünnung aus erwähnten Lös.	10	20	40	60	160	Kontr.
Meerbrasse..	Aqua dest....	{ Aqua dest....	+	+	+	+	—	—
		NaCl-Lös....	+	+	+	±	—	—
	NaCl-Lös....	{ Aqua dest....	+	+	+	+	—	—
		NaCl-Lös....	+	+	+	+	—	—
Scholle ....	Aqua dest....	{ Aqua dest....	+	+	+	—	—	—
		NaCl-Lös....	+	+	+	—	—	—
	NaCl-Lös....	{ Aqua dest....	+	+	+	—	—	—
		NaCl-Lös....	+	+	+	—	—	—
Karpfen ....	Aqua dest....	{ Aqua dest....	+	+	—	—	—	—
		NaCl-Lös....	+	+	+	±	—	—
	NaCl-Lös....	{ Aqua dest....	+	+	+	±	—	—
		NaCl-Lös....	+	+	+	—	—	—
Karausche ..	Aqua dest....	{ Aqua dest....	+	+	—	—	—	—
		NaCl-Lös....	+	+	±	—	—	—
	NaCl-Lös....	{ Aqua dest....	+	+	+	+	—	—
		NaCl-Lös....	+	+	+	—	—	—

Resultat des Versuchs: Bazillen werden mit Fischfleisch-Extrakt agglutiniert.

#### E. Agglutinationsversuch mit dem Extrakt von Rind- oder Pferdefleisch.

Die Versuchsmethode ist ganz gleich der des oben erwähnten Versuchs, sodass sich die Beschreibung derselben erübrigkt. Die Prozente des Eiweisses dieser Fleischarten sind (nach NUKATA).

Arten des Fleisches	Eiweissgehalt (%)
Rindfleisch .....	21,90
Pferdefleisch .....	21,71

Die Resultate des Versuchs sind aus Tabelle 18 ersichtlich.

TABELLE 18.

Agglutinationsversuch mit dem Extrakt von Rind- oder  
Pferdefleisch durch Kolibazillen.

	Arten der Lös.	Wärme-grad.	Wärme-zeit.	5	10	15	20	30	40	60	Kontr.
Rindfleisch	Aqua dest.	55	10'	+	+	-	-	-	-	-	-
		65	10'	+	-	-	-	-	-	-	-
		75	10'	+	-	-	-	-	-	-	-
	NaCl-Lös..	55	10'	+	+	+	+	+	+	+	-
		65	10'	+	-	-	-	-	-	-	-
		75	10'	+	+	+	+	+	+	+	-
Pferdefleisch	Aqua dest.	55	10'	+	+	+	+	+	+	+	-
		65	10'	-	-	-	-	-	-	-	-
		75	10'	-	-	-	-	-	-	-	-
	NaCl-Lös..	55	10'	+	+	+	+	+	+	+	-
		65	10'	+	+	+	+	+	+	+	-
		75	10'	+	-	-	-	-	-	-	-

Bemerkung: Aus anderen Experimenten ergab sich die Notwendigkeit erwärmter Flüssigkeiten. Die Temperaturen und Zeiten sind in obiger Tabelle angegeben.

Resultat des Versuchs: Bazillen werden mit dem Extrakt von Rind- oder Pferdefleisch agglutiniert.

Aus oben erwähnten Resultaten kommt man also zum Schlusse, dass die Bazillen mit verschiedenen Arten Eiweiss, Pepton, und Hämoglobin agglutinierbar sind.

Die obigen Ergebnisse auf meine Versuche mit Rotz-, Koli- und Typhusbazillen ausgedehnt, ohne behaupten zu wollen, dass sie in allen Fällen übereinstimmen, scheinen mir doch eine mehr oder weniger deutliche Auffassung des Wesens des Normalagglutinin zu ermöglichen.

Während die Bazillen auch mit anderen Arten Eiweiss ausser dem Normalserum agglutinierbar sind, und zum Eintritt der Agglutination mit Normalagglutinin eine minimale Menge Salz notwendig ist, wogegen sonst in wirksamen Konzentrationen der Eintritt der Reaktion gehemmt ist, sowie

Normalagglutinin keine Spezifität hat, hat Immunagglutinin indessen die Spezifität, kann aber seine vollständige Wirkung nicht entfalten, ohne Anwesenheit von Salz. Aus diesen Ergebnissen kann man kaum annehmen, dass Normal- und Immunagglutinin, wie bei anderen Antikörpern, identisch sind, wie auch WASSERMAN, (15) SCHELLER (16) und auch EISENBERG (18) dafür halten.

Wenn Normalagglutinin mit der NaCl-Lösung in ausserordentlich minderer Konzentration als 0,85% verdünnt wird, nimmt ein Agglutinationstiter in höherem Grade zu, und es scheint hierbei sich Globulin auszulösen und die Grösse des Emulsoides der Kochsalzmenge entsprechend zu sein.

### 3. ÜBER DIE VERBINDUNG VON AGGLUTININEN UND AGGLUTINOGEN MIT BEZIEHUNG AUF AN- ODER ABWESENHEIT DES SALZES.

Bevor ich die Beschreibung meiner Experiment angebe, referiere ich zunächst die von Joos, nach welchem die Agglutinationsvorgänge in zwei Phasen geteilt werden :

1. Fixierung von Agglutininen und agglutinierenden Substanzen. 2. Ausflockung von Salz u. beladenen Mikroben. Joos gab die Erklärung, dass die agglutinierenden Substanzen und die agglutinierbaren für einander eine starke Affinität besitzen. Ohne Salze vereinigen sie sich auf einander; doch zeigt sich diese Einigung durch kein äusseres Merkmal. Man kann selbst Dosen von Serum beifügen, welche zwei oder drei Mal stärker sind als jene, welche unter Umständen die Agglutination hervorruft, ohne dass die geringste Spur von agglutinierender Substanz in der filtrierten Flüssigkeit entdeckt werden kann: Es ist jedoch eine Grenze vorhanden, über welche hinaus die agglutinierende Substanz in das Filtrat eindringt. Eine bestimmten Menge von Mikroben kann sich nur mit einer begrenzten Quantität von Serum verbinden; sind die Bakterien gesättigt, so bleibt das, was man noch hinzufügt, ohne Verwendung. So erleiden Bakterien, welche eine beträchtliche Menge agglutinierender Substanz gebunden haben, unter der Wirkung derselben keine Modifikation.

Ich habe einige Zweifel für die Erklärung von Joos, und um die Bedeutung des Salzes auf die Agglutination zu erweisen, stellte ich die folgenden Versuche an.

A. Bindungsversuch mit Bazillen und Agglutininen in Beziehung zur An- oder Abwesenheit von Salz.

Versuchsmethode: Rotz-Immungpferdeserum und Typhus-Immunkaninchenserum werden zu diesen Versuche verwandt. Immunsera werden auf 100 fach mit destilliertem Wasser und 0,85%iger NaCl-Lösung verdünnt, zu 5 ccm. beider Flüssigkeiten in Spitzgläsern wird eine halbe Menge einer bei 37°C. gehaltenen 24-stündigen Typhuskultur auf Agar aufgeschwemmt. Nach Verlauf von 2 Stunden bei 37°C. werden die durch einstündige Zentrifugierung erhaltenen klaren Flüssigkeiten mit 0,85%iger NaCl-Lösung stufenweise verdünnt, alle Reagenzgläser werden auf das gleiche Volumen von 1 ccm. gebracht, zu denen zwei Tropfen aus den erwähnten Bakterienaufschwemmungen zugefügt werden. Nach dem bestimmten Zeitraum wird dann das Urteil der Reaktionen gestellt, wie Tabelle 19 zeigt.

TABELLE 19.

Absorptionsversuch mit Immunserum in der Verdünnung mittels destillierten Wassers oder 0,85%iger NaCl-Lösung.

Mit Rotz-Immungpferdeserum und Rotzbazillen.

	Verdünnung Lös. z. Absorption	200	400	800	1600	3200	4600	12800	Kontr.
Agglutinationstiter des Im.-S.....	NaCl-Lös..	++	++	++	++	+	+	—	—
Iste Absorption ..	{ NaCl-Lös.:	±	—	—	—	—	—	—	—
	Aqua dest.	++	++	++	+	+	—	—	—
2te Absorption....	{ NaCl-Lös..	—	—	—	—	—	—	—	—
	Aqua dest.	++	++	++	+	+	—	—	—
3te Absorption....	{ NaCl-Lös..	—	—	—	—	—	—	—	—
	Aqua dest.	++	+	—	—	—	—	—	—

Mit Typhus-Immunkaninchenserum und Typhusbazillen.

	Verdünnung Lös. z. Absorption.	200	400	800	1600	3200	6400	12800	Kontr.
Agglutinationstiter des Im.-S. ....	NaCl-Lös..	++	++	++	++	++	+	+	-
Iste Absorption ..	{ NaCl-Lös..	±	±	-	-	-	-	-	-
	Aqua dest.	++	+	+	+	-	-	-	-
2te Absorption....	{ NaCl-Lös..	-	-	-	-	-	-	-	-
	Aqua dest.	+	+	+	-	-	-	-	-
3te Apsorbtion....	{ NaCl-Lös..	-	-	-	-	-	-	-	-
	Aqua dest.	+	±	-	-	-	-	-	-

Obgleich Immunsera mit destilliertem Wasser oder 0,85%iger NaCl-Lösung verdünnt werden, werden die nach den Absorptionsvorgängen durch Abzentrifugierung erhaltenen klaren Flüssigkeiten mit 0,85%iger NaCl-Lösung stufenweise immer weiter verdünnt, um den Agglutinationstiter zu bestimmen.

Beurteilung der Resultate: Aus oben erwähnten Resultaten, die den durch Absorptionsvergänge verminderte Agglutinationstiter gezeigt haben, wobei der eine in der Verdünnung mit destilliertem Wasser höher nach der Absorption als der andere in der Verdünnung mit 0,85%iger NaCl-Lösung ist, ergibt sich also, dass es vorkommt, wie schon Joos betonte, dass Bazillen und vereinigte Agglutinine ohne Salz sich mit einander verbinden, aber es scheint hierbei der Mechanismus der Bindungsvergänge etwas schwach zu sein.

#### B. Absorptionsversuch mit Agglutininen im Immunserum.

Verwendet wird ein Rotz-Immunkörperdeserum, teils nach vorhergehender Absorption vom Normalagglutinin durch Kolibazillen, teils ohne Vorbehandlung.

Versuchsmethode: Ein Rotz-Immunkörperdeserum wird 50 fach mit 0,85%igen und 0,05%igen NaCl-Lösungen verdünnt, zu 5 ccm. beider Flüssigkeiten in Spitzgläsern wird eine halbe Menge von auf 37°C. gehaltenen 24 stündigen Koli- oder Rotzkulturen auf Agar aufgeschwemmt, nach Verlauf von 2 Stunden bei 37°C. werden die durch einstündige Zentrifugierung erhaltenen klaren Flüssigkeiten mit entsprechenden NaCl-Lösungen stufenweise verdünnt

und alle Reagenzgläser auf das gleiche Volumen von 1 ccm. gebracht, zu denen zwei Tropfen aus erwähnten Bakterienaufschwemmungen zugefügt werden, und dann werdem nach dem bestimmten Zeitraum die Reaktionen beurteilt, wie Tabelle 20 zeigt.

TABELLE 20.

Welche Agglutinine in dem Immunserum wirken hauptsächlich gegen Antigen?

Absorptions-vorgang.			NaCl-Lös.	100	200	400	800	1600	3200	6400	Kontr.
		Rotbaz.-Aufschw. (2 Tropfen)	{ 0,85 % { 0,05 %	++	++	++	++	++	++	++	—
Rotbaz. $\frac{1}{2}$ Kul.	2. Stunden	wie oben	{ 0,85 % { 0,05 %	±	—	—	—	—	—	—	—
Kolibaz. $\frac{1}{2}$ Kul.	bei 37°C. 2 malige Zentrif. in 30 Minuten.	wie oben	{ 0,85 % { 0,05 %	++	++	++	++	++	+	+	—
Kolibaz. $\frac{1}{2}$ Kul.		Kolibaz.-Aufschw.	{ 0,85 % { 0,05 %	++	++	+	—	—	—	—	—
Rotbaz. $\frac{1}{2}$ Kul.		wie oben	{ 0,85 % { 0,05 %	—	—	—	—	—	—	—	—
		wie oben	{ 0,85 % { 0,05 %	++	+	+	—	—	—	—	—
				10	20	40	80	100	200	400	Kontr.

Beurteilung der Resultate: Obgleich Normalagglutinine durch zum Agglutinogen nicht Bezug habende Kolibazillen absorbiert werden oder nicht, treten doch in jedem Falle Immunagglutinationstiter gegen Rotbazillen ein, und obwohl Immunagglutinine durch Rotbazillen absorbiert werden oder nicht, treten doch Normalagglutinationstiter gegen Kolibazillen in gleicher Weise ein. Aus oben beschriebenen Resultaten scheint hervorzugehen, dass das Immunserum in bestimmten NaCl-Lösungen ein gegen agglutinogene Bazillen wirkendes Agglutinin ist, also ausschliesslich Immunagglutinin.

C. Absorptionsversuch der Agglutinine im Immunserum, mit destilliertem Wasser verdünnt.

Im Anschluss an vorhergehenden Versuche, wird ein Rotz-Immunkörperdeserum verwandt, teils nach vorhergehender Absorption durch Koli- oder Rotzbazillen, teils ohne Vorbehandlung, und dann wird sich aus diesem Versuche ergeben, ob Immun- oder Normalagglutinine überhaupt wirkend sind.

Versuchsmethode: Anstatt der NaCl-Lösung wird destilliertes Wasser bei diesem Versuche verwandt. Das Versuchsresultat ergibt sich aus Tabelle 21.

TABELLE 21.

Absorptionsversuch der Agglutinine im Immunserum, mit destilliertem Wasser verdünnt.

Absorptionsvorgang.		Agglutinationsreaktion.	10	20	40	80	160	320	Kontr.
		Rotzaufschw. (2 Tropfen)	++	++	+	+	--	--	--
		Koliaufschw. (2 Tropfen)	++	++	+	+	--	--	--
Kolibaz. $\frac{1}{2}$ Kult.	2 Stunden bei 37°C.	Rotzaufschw. (2 Tropfen)	++	++	+	+	--	--	--
wie oben	2 malige zentrif. je 30 Minuten.	Koliaufschw. (2 Tropfen)	+	±	—	—	--	--	--
Rotzbaz. $\frac{1}{2}$ Kult.	wie oben	wie oben	++	++	+	+	--	--	--
wie oben		Rotzaufschw. (2 Tropfen)	+	±	—	—	--	--	--

Beurteilung der Resultate: Obgleich Rotz-Immunkörperdeserum durch nicht agglutinogene Kolibazillen absorbiert wird, oder nicht, sind doch die Immunagglutinationstiter dieses Serums gegen Rotzbazillen immer gleich und zwar sind diese Beziehungen zwischen Rotz-Immunkörperdeserum und Kolibazillen auch gleich, wie oben erwähnter Versuch zeigt, so scheint also das im Immunserum gegen agglutinogene Bazillen reagierende Agglutinin in destilliertem Wasser hauptsächlich Immunagglutinin zu sein.

*D. Vergleichungsversuch der Affinität zwischen Normal- und Immunagglutininen.*

Welches, Immun- oder Normalagglutinin, hat höhere Affinität gegen agglutinogene Bazillen? Nach Eisler und Laub (17) verhalten sich die Affinitäten von Normal- und Immunagglutininen gegen agglutinogene Bazillen gleichmäßig und nach Eisenberg und VOLK ist die Affinität des Immunagglutinins stärker als die des Normalagglutinins. Dies zeigt folgender Versuch.

**Versuchsmethode:** Verwandt wird ein mit destilliertem Wasser und NaCl-Lösungen in verschiedenen Konzentrationen verdünntes Rotz-Immungpferdeserum, teils nach vorhergehender Absorption durch Rotzbazillen, teils ohne Vorbehandlung und so erhaltene Flüssigkeiten werden noch einmal zu Agglutinationsversuchen durch Kolibazillen verwandt. Eine genaue Beschreibung der Versuchsmethode zu erwähnten Absorptionsversuchen ist überflüssig, weil keine Verschiedenheit stattfindet. (siehe Tabelle 2 und 21.)

**Beurteilung der Resultate:** Obgleich Immunagglutinine im Rotz-Immungpferdeserum durch agglutinogene Rotzbazillen absorbiert werden oder nicht, zeigt der Normalagglutinationstiter dieses Serums gegen Kolibazillen doch keine Verschiedenheit, wohl aber scheint es hierbei, dass Immunagglutinin gegen Agglutinogen höhere Affinität besitzt als Normalagglutinin, obwohl ich noch einige zweifel darüber hege.

*E. Absorptionsversuch mit Normalagglutinin bei An- oder Abwesenheit von Salz.*

Dem Absorptionsversuch mit Immunagglutinin entsprechend, welcher schon beim Versuch dieses Abschnitts angegeben wurde, wird nun ein Absorptionsversuch mit Normalagglutinin bei An- oder Abwesenheit des Salzes ausgeführt.

**Versuchsmethode:** Ein Normalpferdeserum wird 10 fach mit destilliertem Wasser, 0,05%igen und 0,85%igen NaCl-Lösungen verdünnt, zu 3 ccm. dieser Flüssigkeiten in Spitzgläsern wird eine Menge von auf 37°C. gehaltener 24 stündiger Rotz- oder Kolikultur auf Agar aufgeschwemmt, nach 2 Stunden bei 37°C. werde 2 Tropfen der erwähnten Bakterienaufschwemmungen zu der durch 30 Minuten lange Abzentrifugierung erhaltenen überstehenden Flüssigkeit zugefügt und dann nach dem bestimmten Zeitraum die Reaktion beurteilt,

wie Tabelle 22 zeigt. Als Kontrolle werden die Normalagglutinationstiter ohne Vorbehandlung gemessen.

TABELLE 22.

Absorptionsversuch mit Normalagglutinin.

Absorptionsvorgang.		Agglutinationsreaktion.	10	20	40	60	80	160	Kontr.
mit Aqua dest verdünnt	ohne Absorp.		++	++	++	+	+	-	-
	Kolibaz.-Aufs. (2 Tropfen)	++	++	++	+	+	-	-	
	Rotzbaz.-Aufs. (2 Tropfen)	++	++	++	+	+	-	-	
	wie oben	++	+	+	+	-	-	-	
	Kolibaz.-Aufs. (2 Tropfen)	++	+	+	-	-	-	-	
	Kolibaz. ( $\frac{1}{2}$ K.)	wie oben	+	+	+	-	-	-	-
		Rotzbaz. ( $\frac{1}{2}$ K.)	+	+	+	-	-	-	-
		Kolibaz.-Aufs. (2 Tropfen)	++	++	++	+	+	-	-
		wie oben	++	+	+	+	-	-	-
		Rotzbaz.-Aufs. (2 Tropfen)	+	+	+	-	-	-	-
mit 0,85% NaCl-Lösung verdünnt	ohne Absorp.	Kolibaz.-Aufs. (2 Tropfen)	++	++	++	+	+	-	-
		Kolibaz. ( $\frac{1}{2}$ K.)	++	++	++	+	+	-	-
		Rotzbaz. ( $\frac{1}{2}$ K.)	++	++	++	+	+	-	-
		Kolibaz. ( $\frac{1}{2}$ K.)	++	+	+	+	+	-	-
		Rotzbaz. ( $\frac{1}{2}$ K.)	+	+	+	+	+	-	-
	Kolibaz. ( $\frac{1}{2}$ K.)	Kolibaz.-Aufs. (2 Tropfen)	++	++	++	+	+	-	-
		wie oben	++	+	+	+	+	-	-
		Rotzbaz.-Aufs. (2 Tropfen)	+	+	+	+	+	-	-
		Kolibaz.-Aufs. (2 Tropfen)	++	++	++	+	+	-	-
		Rotzbaz. ( $\frac{1}{2}$ K.)	+	+	+	+	+	-	-
mit 0,05% NaCl-Lösung verdünnt	ohne Absorp.	Kolibaz.-Aufs. (2 Tropfen)	++	++	++	+	+	-	-
		Kolibaz. ( $\frac{1}{2}$ K.)	++	++	++	+	+	-	-
		Rotzbaz. ( $\frac{1}{2}$ K.)	++	++	++	+	+	-	-
		Kolibaz. ( $\frac{1}{2}$ K.)	++	++	++	+	+	-	-
		Rotzbaz. ( $\frac{1}{2}$ K.)	++	++	++	+	+	-	-
	Kolibaz. ( $\frac{1}{2}$ K.)	Kolibaz.-Aufs. (2 Tropfen)	++	++	++	+	+	-	-
		wie oben	++	+	+	+	+	-	-
		Rotzbaz.-Aufs. (2 Tropfen)	+	+	+	+	+	-	-
		Kolibaz.-Aufs. (2 Tropfen)	++	++	++	+	+	-	-
		Rotzbaz. ( $\frac{1}{2}$ K.)	+	+	+	+	+	-	-

Beurteilung der Resultate: Obgleich das Salz beim Agglutinationsversuch reichlich vorhanden ist oder nicht, treten doch die Normalagglutinationstiter deutlich niedriger hervor, als wenn die Reaktionen durch eigene oder beziehungslose Bazillen mit dem mittels destillierten Wessers oder einer NaCl-Lösung in bestimmten Konzentrationen verdünnten Normalpferdeserum ausgeführt werden; aus oben erwähnten Resultaten kommt deutlich zum Ausdruck, dass das Normalagglutinin keine Spezifität für eine der beiden Arten von Bazillen hat.

F. Die Beziehung der Absorptionsmenge des Immunagglutinins zu den quantitativen Verhältnissen des Salzes.

Die Verschiedenheit der Salzmenge bei den Agglutinationsversuchen spielt eine wichtige Rolle zum Bindungsvermögen zwischen agglutinablen und agglutinierbaren Substanzen, wie Versuch A zeigt. Dennoch wird dieser Versuch weiter ausgeführt.

Versuchsmethode: Ein Rotz-Immumpferdeserum wird mit NaCl-Lösungen in verschiedenen Konzentrationen 100 fach verdünnt, zu 3 ccm. dieser Flüssigkeiten in Spitzgläsern wird eine Menge der auf 37°C. gehaltenen 20-stündigen Rotzkultur auf Agar aufgeschwemmt, nach 2 Stunden bei 37°C. werden die durch 30 Minuten lange Abzentrifugierung erhaltenen überstehenden Flüssigkeiten mit 0,85%iger NaCl-Lösung stufenweise verdünnt, und alle Reagenzgläser auf das gleiche Volumen von 1 ccm. gebracht, zu denen 2 Tropfen von in 0,85%iger NaCl-Lösung aufgeschwemmten Rotzbazillen zugefügt werden und dann werden nach dem bestimmten Zeitraum die Reaktionen beurteilt, wie Tabelle 23 zeigt.

TABELLE 23.

Absorptionsversuch (Rotzbazillen).

Verdünnung des Immunserums.	100	200	400	800	1600	3200	6400	12800	Kontroll
Konzentration der NaCl-Lösungen bei der Vorbehandl.	++	++	++	++	+	+	±	-	-

Agglutinationstiter dieses Immunserums.

Agglutinationstiter nach den Absorptionsvorgängen.

	100	200	400	800	1600	3200	6400	12800	Kontr.
0,85 % .....	+	±	—	—	—	—	—	—	—
0,60 „ .....	+	±	—	—	—	—	—	—	—
0,40 „ .....	+	±	—	—	—	—	—	—	—
0,20 „ .....	+	±	—	—	—	—	—	—	—
0,09 „ .....	+	±	—	—	—	—	—	—	—
0,07 „ .....	+	±	—	—	—	—	—	—	—
0,05 „ .....	+	+	±	—	—	—	—	—	—
0,03 „ .....	+	+	±	—	—	—	—	—	—

Beurteilung der Resultate : Die ganze Beweisführung muss von der Untersuchung ausgehen, dass zum passenden Eintritt der Agglutination die Anwesenheit des Salzes notwendig ist, aber nicht so viel wie 0,85%.

### Zusammenfassungen.

1. Zum Eintritt der vollständigen Wirkung des Immunagglutinins ist die Anwesenheit einer bestimmten Salzmenge notwendig, aber die Beziehungen zwischen dem Eintritt der Agglutination und der Salzmenge sind nicht immer absolut festzustellen.
2. Zum Eintritt der Normalagglutination ist die Anwesenheit des Salzes notwendig, aber eine kleine Salzmenge genügt, um eine vollständige Wirkung zu erzeugen.
3. Die Wirkung des sogenannten Normalagglutinins beruht nicht, wie man früher betonte, auf der Wirkung des spezifischen Rezeptors, aber es scheinen Globulin und Albumin eine wichtige Rolle zu spielen.
4. Ausser mit Serum tritt eine normalagglutinationähnliche Agglutination mit anderen Eiweissen ein.
5. Aus Absorptionsversuchen zu urteilen scheint Normalagglutinin keine wesentliche Spezifität zu behalten, und das Salz mag vorhanden sein oder nicht.

6. Agglutinin kann sich mit dem Bazillus verbinden, wie schon Joos betonte, aber in diesem Falle ist der Mechanismus der Verbindung ziemlich schwach.

7. Den Eintritt der Agglutination bewirkt hauptsächlich das Immunagglutinin, wenn auch das Immunserum mit NaCl-Lösung oder destilliertem Wasser verdünnt wird.

8. Es scheint aber das Immunagglutinin gegen eigenes Agglutinogen höhere Affinität zu besitzen als das Normalagglutinin, wie EISENBERG und VOLK betonen.

9. Die Anwesenheit einer bestimmten Salzmenge ist notwendig, um das Agglutinogen zum Immunagglutinin am vollständigsten aufzunehmen.

Zum Schlusse erlaube ich mir, meinem hochverehrten Chef, Herrn Prof. Dr. HAYASHI, sowie meinen hochverehrten Lehrern, Herrn Prof. Dr. T. YOKOTE, Vorstand der Forschungsabteilung für Bakteriologie und Immunitätslehre und Herrn Assist.-Prof. Dr. TAKENOUCHI, für ihre Anregung und freundliche Anleitung bei der Ausführung dieser Arbeit meinen ergebensten Dank auszusprechen.

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# Ueber die proagglutinoidähnliche Reaktion durch Hämoglobinlösung.

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## Einleitung.

Beim Agglutinationsversuch mit lang konservierten Immunsera beobachtet man häufig, dass manche der verwandten Immunsera bei den schwächsten Verdünnungen keine Agglutination, oder nur eine mehr oder weniger unvollständige zeigen, bei anderen dagegen tritt dieses Phänomen nicht ein, was schon mehrere Autoren bemerkt haben und SHIGA (5) als Proagglutinoid-Reaktion bezeichnet hat.

Im ersten Falle fanden EISENBERG und VOLK (1) dieses Phänomen bei Typhusserum, LIPSTEIN (2) und SCHWONER (3) bei Diphtherieserum, A. WASSERMANN (4) bei Choleraserum, SHIGA bei Dysenterieserum. Ferner beobachtet Lipstein, dass ein frisches Diphtherieserum durch einen Stamm der Diphtheriebazillen dieses Phänomen auch erzeugt

Der Verfasser (6) fand, dass wenn Rotzbazillen durch Hämoglobinlösungen aus normalen Tieren agglutiniert werden, solches Phänomen immer erzeugt wird, was schon mehrere Autoren als Proagglutinoid-Reaktion durch Immunsera bezeichneten. Da mir diese Tatsache für die Serologie interessant zu sein scheint, will ich meine diesbezüglichen Versuche beschreiben.

Die Hämoglobinlösungen aus anderen Tieren (Kaninchen, Meerschweinchchen) mögen natürlich dieses Phänomen auch erzeugen, aber ich habe besonders die Ziegenhämoglobinlösung bei meinen Versuchen verwandt, weil sie diese Reaktionen am stärksten hervorruft.

### Meine Versuche.

#### MATERIAL DER VERSUCHE.

1. Rotbazillenaufschwemmung: Eine Menge einer auf 37°C. gehaltenen 24-stündigen Rotzkultur auf Agar, welche vorher einige Jahren zu verschiedenen Versuchen verwandt wurde, wird in 10 ccm. destilliertem Wasser oder NaCl-Lösungen in verschiedenen Konzentrationen aufgeschwenmt.

2. Ziegenhämoglobinlösung: Defibriniert wird ein Ziegenblut, dessen brauchbare Menge aus der Jugularvene der in diesem Institut gehaltenen normalen Ziege entnommen ist. Das durch Zentrifugierung ausgeschiedene Serum wird möglichst aufgesaugt, die so erhaltenen Blutkörperchen werden 5 mal mit 0,85% iger NaCl-Lösung gewaschen. Mit den nach der letzten Waschung aus der NaCl-Lösung möglichst abgetrennten Blutkörperchen bekommt man mit 20 fachem destillierten Wasser Hämolyse. Dann wird das Stroma durch Zentrifugierung niedergeschlagen und die überstehende Hämoglobinlösung zu diesen Versuchen verwandt.

#### AGGLUTINATIONSVERSUCH MIT ZIEGENHÄMOGLOBINLÖSUNG DURCH ROTBAZILLEN.

Versuchsmethode: Oben erwähnte Ziegenhämoglobinlösung wird stufenweise mit destilliertem Wasser oder NaCl-Lösungen in verschiedenen Konzentrationen verdünnt, wie folgende Tabelle zeigt, und alle Reagenzgläser werden zu 1 ccm. aufgefüllt. Danach werden 2 Tropfen der Rotbazillenaufschwemmung in alle Reagenzgläser gegeben und durch Schüttelung gemischt. Die Resultate werden nach 24 Stunden bei 37°C. beobachtet, wie Tabelle 1 zeigt.

TABELLE 1.

Agglutinationsversuch durch Rotbazillen mit Ziegenhämoglobinlösung.

Lös. zur Verdünnung der Hämog.-lösung.	10	20	40	80	160	320	640	1280	2560	5120	10240	20480	40960	Kontrolle
Aqua dest .....	+	+	+	+	+	+	+	+	+	+	+	+	+	—
0,01% .....	+	+	+	+	+	+	+	+	+	+	+	+	—	—
0,02% .....	+	+	+	+	+	+	+	+	+	+	+	+	+	—
0,03% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,04% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,05% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,06% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,07% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,08% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,09% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,1% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,2% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,3% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,4% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,5% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,6% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,7% .....	+	+	+	++	++	++	++	++	++	++	++	++	+	—
0,85% .....	+	+	+	+	+	++	++	++	++	++	—	—	—	—
1,0% .....	+	+	+	+	+	++	++	+	—	—	—	—	—	—
2,0% .....	+	+	+	+	+	+	++	—	—	—	—	—	—	—

SHIGA beobachtet, dass Proagglutinoid-Reaktion bei Dysenterieserum die in gewöhnlicher Weise eintritt, verschwindet, wenn beim Agglutinationsversuch in eigenartiger Weise eine 5 fache Menge von Bakterien verwandt wird.

SHIGA's Beobachtung entsprechend habe ich untersucht, ob dieses Verhältnis zwischen Rotbazillen und Ziegenhämoglobinlösung zutage tritt oder nicht. Zu diesem Zwecke habe ich die Abschwächung des Eintritts der proagglutinoidähnliche Reaktion und die Verminderung des Agglutinationstiters beobachtet, wie Tabelle 2 zeigt.

TABELLE 2.

Derselbe Versuch mit 5 fach konzentrierter Bazillenaufschwemmung.

Lös. zur Verdünnung der Hämoglobinlösung.	10	20	40	80	160	320	640	1280	2560	5120	10240	20480	Kontrolle
Aqua dest .....	+	+	+	#	#	+	-	-	-	-	-	-	-
0,01% .....	+	+	+	++	++	++	+	-	-	-	-	-	-
0,02% .....	+	+	+	++	++	++	+	-	-	-	-	-	-
0,03% .....	+	+	++	##	##	##	##	-	-	-	-	-	-
0,04% .....	+	+	++	##	##	##	##	-	-	-	-	-	-
0,05% .....	+	+	++	##	##	##	##	-	-	-	-	-	-
0,06% .....	+	+	++	##	##	##	##	-	-	-	-	-	-
0,07% .....	+	+	++	##	##	##	##	-	-	-	-	-	-
0,08% .....	+	+	++	##	##	##	##	-	-	-	-	-	-
0,09% .....	+	+	++	##	##	##	##	-	-	-	-	-	-
0,1% .....	+	+	++	##	##	##	##	-	-	-	-	-	-
0,2%	+	+	++	##	##	##	##	-	-	-	-	-	-
0,3%	+	+	+	##	##	##	##	-	-	-	-	-	-
0,4%	+	+	+	##	##	##	##	-	-	-	-	-	-
0,5%	+	+	+	+	##	##	##	-	-	-	-	-	-
0,6%	+	+	+	+	##	##	##	-	-	-	-	-	-
0,7%	+	+	+	+	##	##	##	-	-	-	-	-	-
0,85%	+	+	+	+	##	##	##	-	-	-	-	-	-

Beurteilung der Resultate: Wenn man beim Agglutinationsversuch Ziegenhämoglobinlösung und Rotzbazillen verwendet, so kann man eine proagglutinoidähnliche Reaktion erzeugen.

### Einige Hypothesen zur Erklärung der Proagglutinoidreaktion.

Es gibt mehrere Hypothesen zur Erklärung der Proagglutinoidreaktion: EISENBERG und VOLK betonen, dass in diesem Falle die Bakterien vermöge der grossen Avidität sich zuerst mit den unwirksamen Agglutinoiden sättigen und in stärkeren Verdünnungen, welche keine oder nur geringe Mengen dieser

Stoffe beherbergen, umgekehrt prompte Agglutination ergeben. Nach Lipstein hat ein bestimmter Complex „a“ des Bacterium im Tierkörper zu ganz verschiedenen Rezeptoren eine besondere Verwandtschaft, aber nur die Beziehung der Rezeptoren zweiter Ordnung gibt, nach den bisherigen Anschauungen, Anlass zur Entstehung eines Agglutinins, während die Rezeptoren erster oder dritter Ordnung nicht in dieser Richtung wirken. Bei geeigneten Aviditätsverhältnissen werden aber diese, Agglutination nicht hervorrufenden Zellabkömmlinge in der Form hemmender Substanzen in die Erscheinung treten. Man konnte diese Substanzen ihrer Wirkung nach als „falsche Antiagglutinine“ bezeichnen. A. WASSERMANN nimmt an, dass in diesem Falle ein sehr unregelmässiges Verhalten des Serums sich ergeben muss, und je nachdem zufälliger Weise mehr oder weniger Bakterien Agglutinin oder Agglutinoid binden, die Agglutination eine mehr oder weniger unvollständige sein wird. Eine vollständige Agglutination kommt überhaupt nicht zustande, selbst bei den schwächsten Verdünnungen nicht, indem stets ein Teil der Bakterien sich mit Agglutinoiden verbindet. ASAKAWA (7) studierte dieses Phänomen, das er schon als ein umgekehrt sich verhaltendes Phänomen bezeichnet, und er nimmt den Mangel an der haptophoren Gruppe des Bacteriums als Ursache an, dabei seien die Bindungsmengenverhältnisse der Agglutinine zu denselben Bazillen also gering, und in den grösseren Quantitäten des Serums, welches bei schwächsten Verdünnungen einen hohen Grad von Viskosität zeigt, werden die Bazillen umgekehrt gar nicht agglutiniert.

SHIGA betont, es handle sich dementsprechend um die Wirkung von Körpern, welche durch äussere Eingriffe aus dem Agglutinin entstehen, welche weiterhin eine höhere Avidität zu den Bazillen haben, als das unveränderte Agglutinin, und er nennt diese Phänomene Proagglutinoid-Reaktion, wie oben erwähnt.

### Meine Erklärung zur proagglutinoidähnlichen Reaktion.

Aus oben erwähnten Resultaten meiner Versuche kommt man zu einer anderen Erklärung für den Eintritt der proagglutinoidähnlichen Reaktion. Wird eine mit stark kochsalzhaltigen Lösungen verdünnte Hämoglobinlösung verwandt, dann ist der Agglutinationstiter niedrig und die Reaktion selbst

auch schwach. Aber je passender die Konzentration der angewandten NaCl-Lösung wird, desto höher und deutlicher werden die Titer und solche Reaktionen bei Agglutination.

Von diesen Tatsachen aus scheint es mir, dass die Beziehung zwischen dem Agglutinationstiter oder der proagglutinoidähnlichen Reaktion mit Hämoglobinlösung und der Menge des Kochsalzes den wichtigen Anlass zur Entstehung des Erfolges gibt. Mit anderen Worten: es ist notwendig zum vollständigen Eintritt dieses Phänomens, dass die Grösse der Molekulären aus Emulsoiden und die Konzentrationen der Flüssigkeiten passend sind.

Je nachdem die Menge des Kochsalzes in den Flüssigkeiten sich verändert, werden die Grösse der Molekulären aus Emulsoiden sich auch verändern, was ein schon bekanntes Gesetz ist.

Obschon die Grösse der Molekulären passend ist, wird der vollständige Eintritt der Agglutination nicht zustande kommen, wenn die Konzentration der Flüssigkeit für das Bindungsvermögen nicht geeignet ist.

Emulsoide selbst, ohne Salz, haben wenig Avidität zu Bazillen, sodass in sonst wirksamen Konzentrationen keine Agglutination mehr eintritt.

Ich habe schon die Rolle des Salzes bei der Agglutination studiert, und gefunden, dass das Salz zum Eintreten der Agglutination eine wichtige Rolle spielt, wie Joos betont. Emulsoide, schwache Avidität zu Bazillen besitzend, wendet sich zuerst nach denselben mittels Hilfe des Salzes, resp. Natrium-Ion als Katalysator. Wird nun jedoch Hämoglobinlösung minimal verdünnt, so kommt keine Agglutination oder nur eine mehr oder weniger unvollständige zustande, ohne dass das Salz seine Wirkung ausüben kann.

Ich schiebe die Erforschung der Bedeutung des Mechanismus später auf, sowie auch die Frage ob meine proagglutinoidähnliche Reaktion mit Hämoglobinlösung und die Proagglutinoid-Reaktion mit Immunsera der anderer Autoren identisch sind oder nicht.

Zum Schlusse ist es mir eine angenehme Pflicht, meinen hochverehrten Lehrern, Herrn Prof. Dr. C. YOKOTE, Herrn Prof. Dr. H. HAYASHI, und Herrn Assist.-Prof. Dr. M. TAKENOUCHI sowie Herrn Priv.-Doz. Dr. T. KOMOTO, für ihre ständige Leitung und Unterstützung bei der Ausführung dieser Arbeit meinen herzlichen Dank auszusprechen.

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# Weitere Studien über die Bedeutung des Salzes bei der Agglutination und ihre Anwendung zur Serodiagnostik des Rotzes.

VON

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## **Einleitung.**

Unter den Serodiagnostiken des Rotzes, welcher sich relativ schnell und genau diagnostizieren lässt, zeichnet sich der Agglutinationsversuch vor allem durch eine besondere Bedeutung aus und ist deshalb zur Serodiagnostik auf diesem Felde mehrfach benutzt worden.

Jedoch gibt es eine Schwierigkeit in der Beurteilung der Rötz-Diagnose mittelst der Agglutination, indem das Normalpferdeserum selbst durch eigenes Normalagglutinin in stärksten Verdünnungen hohen Agglutinationstiter d.i. hohe Normalagglutination hervorruft.

Es existieren mehrere Arten der Serodiagnostik des Rotzes: Komplementablenkung, Konglutination, K. H. Reaktion u.s.w., aber es scheint mir, dass diese wohl im Laboratorium angewendet werden können, aber nicht an den zahlreichen Pferden auf dem Felde.

Die Thermoreaktion der subkutanen Malleinisation ist nicht zuverlässig; ich sowie meine Senioren fanden, dass unsere Experimente durch diese Methode immer unvollständige Resultate ergaben.

Ophthalmoreaktion mit Mallein gibt uns etwas genauere Resultate als subkutane Malleinisation; außer den stark positiven oder stark negativen Resultaten scheint es uns, dass diese auch mehr oder weniger die zweifelhaften angibt, ohne dass die individuelle Beurteilung zu sehr in den Vordergrund tritt.

Bei der Anwendung der Agglutination zur Serodiagnostik des Rotzes fand man häufig bei gesunden Pferden die Normalagglutination bei Verdünnung 1: 200-1 : 700 (WLADIMIROFF(3), BOURGES u. MÉRRY(4), POKCHICHEVSKY(5), AFFANASIEFF, MOOR u. TAYLOR). Nach HUTYRA(2) ergeben sich bei negativem sowie positivem Ausfall der Probe 20% Fehlerdiagnosen.

ANDREJEW(1), der sich bemühte, durch Absorption und Filtrationsversuch sowie Erhitzen Unterschiede zwischen den normalen und immunisatorischen Rotzagglutininen zu finden, erhielt doch keine deutlich regelmässigen Resultate zwischen den Sera der normalen Pferde und den rotzkranken.

Ergeben sich diese Resultate immer, wie HUTYRA u.s.w. betonen, so ist die Rotzdiagnose durch Agglutination unsicher. Wenn man durch gewisse Methode einen Unterschied zwischen den normalen und immunisatorischen Rotzagglutininen finden könnte, so würde der Wert des Agglutinationsversuchs bei der Rotzdiagnose grösser werden.

In meinem früheren Experiment über die Bedeutung des Salzes bei Agglutination habe ich die Verschiedenheit des Wesens zwischen Normal- und Immunagglutininen ziemlich genau studiert und es zeigt sich der maximale Agglutinationstiter der Sera infolge der Arten des Agglutinins verschieden, je nachdem die Versuchssera durch verschiedene Konzentrationen der NaCl-Lösung verdünnt werden, obwohl Immunagglutinin höhere Affinität zum entsprechenden Agglutinogen hat als Normalagglutinin, wie Eisenberg und Volk betonen.

Vom oben erwähnten Standpunkte aus können wir das Vorhandensein des Immunagglutinins im Serum erkennen, wenn der eine Agglutinationstiter immer höher ist als der andere, falls man zwei Reihen Agglutinationsversuche ausführt. In meinen bisherigen Experimenten bei Rötz-Agglutination beobachtete ich, dass der Normalagglutinationstiter durch ein mit 0,03%iger NaCl-Lösung verdünntes Pferdeserum immer höher als durch ein mit 0,85%iger NaCl-Lösung verdünntes. Findet sich diese Beziehung immer, so kann man den Agglutinationswert der Serodiagnostik beim Rötz schätzen, so dass die bisherige unvollständige Reaktion verbessert wird.

### Meine Versuche.

#### 1. MATERIAL DER VERSUCHE.

1. Rotbazillenaufschwemmung: Eine Menge einer auf 37°C. gehaltenen 24 stündigen Rotzkultur auf Agar, welche vorher einige Jahren zu verschiedenen Versuchen verwandt wurde, wird in 10 ccm. destilliertem Wasser oder NaCl-Lösungen in verschiedenen Konzentrationen aufgeschwemmt.

2. Versuchspferde: Alle sind gesund und gehören der Schule für Militär-Veterinäre.

3. Normalpferdeserum: Ein Serum, vom aus der Vena jugularis entnommenen Blut ausgeschieden, wird verwandt. Die Tiere gehörten diesem Institut an.

4. Rotzinfiziertes Pferdeserum: Ein Serum, welches mir Herr KOBAYASHI, Chef des Veterinärkorps der 17ten Division in Mandschurei, und Herr ABE, Generaloberveterinär in der Kavallerie-Abteilung des Kriegsministeriums, zustellen, ist aus natürlich infizierten chinesischen Pferden entnommen und mit 0,5%igem Karbol gemischt.

#### 2. VERSUCHE.

1) Welchen Prozentsatz der NaCl-Lösungen müssen wir Agglutinationsversuche durch Rotbazillen mit Rotzimmunpferdeserum brauchen, zur Serumverdünnung, um maximalen Titer des Serums zu bestimmen?

Der Zweck dieses Versuchs ist zu bestätigen, dass beim Agglutinationsversuch durch Rotbazillen mit dem mit 0,85%iger oder einer gewissen NaCl-Lösung verdünnten Serum der Agglutinationstiter des mit einer gewissen NaCl-Lösung verdünnten Serums immer höher ist als der des mit 0,85%iger NaCl-Lösung verdünnten, wenn das Pferd gesund ist, dass aber der Agglutinationstiter des mit 0,85%iger NaCl-Lösung verdünnten Serums anderseits immer niedriger ist als der des mit einer gewissen NaCl-Lösung verdünnten, wenn das Pferd natürlich oder künstlich infiziert ist.

Versuchsmethode: Den Versuchspferden, Kanjyo, Tosen und Yakei, wurde am 11-Jan. 1917 eine Menge von 1/5 Normalöse der bei 60°C. 30 Min. lang erhitzten Rotzkultur auf Agar intravenös eingespritzt, und eine

Entblutung wurde am 9 Tage nach der Impfung ausgeführt und dann das Serum ausgeschieden. So erhaltenes Serum wird stufenweise von 20 fach abwärts mit destilliertem Wasser oder NaCl-Lösung in verschiedenen Konzentrationen verdünnt, wie Tabelle 1 zeigt, und alle Reagenzgläser werden auf 1 cem. aufgefüllt, danach werden zwei Tropfen der Bakterienaufschwemmung in alle Reagenzgläser gegeben und durch Schütteln gemischt. Das Resultat des Versuchs wird nach 24 Stunden bei 37°C. beobachtet, wie Tabelle 1 zeigt.

TABELLE 1 (a).

(Versuchspferd Tosen).

Agglutinationsversuch durch Rotzbazillen mit dem mittelst destillierten Wassers oder NaCl-Lösungen in verschiedenen Konzentrationen verdünnten Rott-Immungpferdeserum.

Aus diesen Versuchsresultaten scheint der Agglutinationstiter am höchsten bei der Verdünnung des Rott-Immunkrohndeserums mit 0,1%iger NaCl-Lösung, wenn dasselbe mit verschiedenen NaCl-Lösungen verdünnt wird.

Zur Kontrolle und der Zweckmässigkeit halber beschreibe ich nun meinen Versuch der Normalagglutination durch Rotbazillen, der schon in meiner anderen Abhandlung erwähnt wurde. In diesem Falle ist der Agglutinationstiter am höchsten bei der Verdünnung des Normalpferdeserums mit 0,05%iger NaCl-Lösung, wie Tabelle 2 zeigt.

TABELLE 1 (b).

(Versuchspferd Kanjyo).

Verdünnung des Serums. Lösung zur Verdünnung.	20	40	80	160	320	640	1080	2560	5120	10240	20480	Kontrolle
0,85 %.....	#	#	#	#	#	#	#	+				
0,7 „.....	#	#	#	#	#	#	#	+	+			
0,6 „.....	#	#	#	#	#	#	#	+	+			
0,5 „.....	#	#	#	#	#	#	#	+	+			
0,4 „.....	#	#	#	#	#	#	#	+	+			
0,3 „.....	#	#	#	#	#	#	#	+	+			
0,2 „.....	#	#	#	#	#	#	#	+	+			
0,1 „.....	#	#	#	#	#	#	#	+	+			
0,09 „.....	#	#	#	#	#	#	#	+	+			
0,08 „.....	#	#	#	#	#	#	#	+	+			
0,07 „.....	#	#	#	#	#	#	#	+	+			
0,06 „.....	#	#	#	#	#	#	#	+	+			
0,05 „.....	#	#	#	#	#	#	#	+	+			
0,04 „.....	#	#	#	#	#	#	#	+	+			
0,03 „.....	#	#	#	#	#	#	#	+	+			
0,02 „.....	#	#	#	#	#	#	#	+	+			
0,01 „.....	#	#	#	#	#	#	#	+	+			
Aqua dest.....	#	#	#	#	#	#	#	+	+			

TABELLE 1 (c).

(Versuchspferd Yakei).

Verdünnung des Serums. Lösung zur Verdünnung.	20	40	80	160	320	640	1080	2560	5120	10240	20480	Kontrolle.
0,85 % .....	#	#	#	#	#	#	#	#	+	+	+	-
0,7 „ .....	#	#	#	#	#	#	#	+	+	+	+	-
0,6 „ .....	#	#	#	#	#	#	#	+	+	+	+	-
0,5 „ .....	#	#	#	#	#	#	#	+	+	+	+	-
0,4 „ .....	#	#	#	#	#	#	#	+	+	+	+	-
0,3 „ .....	#	#	#	#	#	#	#	+	+	+	+	-
0,2 „ .....	#	#	#	#	#	#	#	+	+	+	+	-
0,1 „ .....	#	#	#	#	#	#	#	+	+	+	+	-
0,09 „ .....	#	#	#	#	#	#	#	+	+	+	+	-
0,08 „ .....	#	#	#	#	#	#	#	+	+	+	+	-
0,07 „ .....	#	#	#	#	#	#	#	+	+	+	+	-
0,06 „ .....	#	#	#	#	+	+	+	+	+	+	+	-
0,05 „ .....	#	#	#	+	+	+	+	+	+	+	+	-
0,04 „ .....	#	#	+	+	+	+	+	+	+	+	+	-
0,03 „ .....	#	#	+	+	+	+	+	+	+	+	+	-
0,02 „ .....	#	#	+	+	+	+	+	+	+	+	+	-
0,01 „ .....	#	#	+	+	+	+	+	+	+	+	+	-
Aqua dest. ....	#	+	+	+	-	-	-	-	-	-	-	-

TABELLE 2.

Agglutinationsversuch mit dem in verschiedenen konzentrierten NaCl-Lösungen verdünnten Normalpferdeserum durch Rotbazillen.

Verdünnung des Serums. NaCl-Lösungen zur Verdünnung des Serums.	10	15	20	40	60	80	120	320	Kontr.
Aqua dest. ....	#	#	#	+	+	-	-	-	-
0,01 % .....	#	#	#	#	+	+	+	+	-
0,02 „ .....	#	#	#	#	#	+	+	-	-

NaCl-Lösungen zur Verdünnung des Serums.	Verdünnung des Serums.									Kontr.
	10	15	20	40	60	80	120	320		
0,03 % .....	#	#	#	#	#	#	-	-	+	-
0,1 „ .....	#	#	#	#	#	#	-	-	-	-
0,2 „ .....	#	#	+	+	+	+	-	-	-	-
0,3 „ .....	#	#	+	+	+	+	-	-	-	-
0,4 „ .....	#	#	+	+	+	+	-	-	-	-
0,5 „ .....	#	#	+	+	+	+	-	-	-	-
0,6 „ .....	#	#	+	+	+	+	-	-	-	-
0,7 „ .....	+	+	+	+	+	+	-	-	-	-
0,85 „ .....	+	+	+	+	+	+	-	-	-	-

NaCl-Lösungen zur Verdünnung des Serums.	Verdünnung des Serums.											Kontrolle.
	200	300	400	600	800	1200	1600	2400	3200	4800	6400	
0,04 % .....	#	#	#	#	#	#	+	+	+	-	-	-
0,05 „ .....	#	#	#	#	#	#	+	+	+	+	+	-
0,06 „ .....	#	#	#	#	#	#	+	+	+	-	-	-
0,07 „ .....	#	#	#	#	#	+	+	+	+	-	-	-
0,08 „ .....	#	#	#	#	+	+	+	+	+	-	-	-
0,09 „ .....	#	#	#	#	+	+	+	+	+	-	-	-

Nach oben beschriebenen Agglutinationsversuchen erscheinen die Agglutinationstiter des Normalpferderesums bei der Verdünnung durch NaCl-Lösungen in verschiedenen Konzentrationen niedriger als die des Immunpferderesums, wenn die beiden Sera mittelst konzentrierter NaCl-Lösung, höher als 0,07%iger, verdünnt werden, demgegenüber scheinen die Agglutinationstiter des Normalpferderesums höher als die des Immunpferderesums, wenn die beiden Sera mittelst konzentrierter NaCl-Lösung, niedriger als 0,07%iger, verdünnt werden.

Durchaus geht hervor, dass, wenn die Immunagglutinationstiter ziemlich höher sind, der Immunagglutinationstiter der Sera am höchsten bei der Verdünnung mittelst 0,1%iger NaCl-Lösung erscheint.

Um meine Annahme noch weiter zu bestätigen, habe ich folgenden Versuch ausgeführt.

Den Versuchspferden, Shunshu, Mizuiwa und Yokoyuwa, wurde am 18 Dez. 1916 eine Menge von 1/5 Normalösse der bei 60°C. 30 Minuten lang erhielten 24-stündigen Rotzkultur auf Agar intravenös eingespritzt. Nach 9 Tagen wurde eine brauchbare Menge von Blut entnommen und das von diesem ausgeschiedene Serum wurde zum Versuch verwandt, wie Tabelle 3 zeigt.

Die Versuchsmethode ist ganz die gleiche wie bei dem oben beschriebenen Versuch, deshalb habe ich die Beschreibung desselben abgekürzt.

Die Resultate des Versuchs sind aus Tabelle 3 ersichtlich.

TABELLE 3.

Agglutinationsversuch mit dem in verschiedenen konzentrierten NaCl-Lösungen verdünnten Immunpferdeserum durch Rotzbazillen.

Verdünnung des Serums. NaCl-Lös. zur Verdünnung des Serums.	10	20	40	80	160	320	640	1280	2560	5120	Kontrolle.
0,85 %.....	++	++	++	+	+	+	+	±	-	-	-
0,7 ,.....	++	++	++	+	+	+	+	±	-	-	-
0,6 ,.....	++	++	++	+	+	+	+	±	-	-	-
0,5 ,.....	++	++	++	+	+	+	+	±	-	-	-
0,4 ,.....	++	++	++	+	+	+	+	+	-	-	-
0,3 ,.....	++	++	++	+	+	+	+	+	-	-	-
0,2 ,.....	++	++	++	+	+	+	+	+	-	-	-
0,1 ,.....	++	++	++	++	++	++	++	+	-	-	-
0,09 ,.....	++	++	++	++	++	++	+	+	-	-	-
0,08 ,.....	++	++	++	++	++	++	+	+	-	-	-
0,07 „.....	++	++	++	++	++	++	+	+	-	-	-
0,06 „.....	++	++	++	++	++	++	+	+	-	-	-
0,05 „.....	++	++	++	++	++	++	+	+	-	-	-
0,04 „.....	++	++	++	++	++	+	+	-	-	-	-
0,03 „.....	++	++	++	+	+	-	-	-	-	-	-
0,02 „.....	++	++	++	+	+	-	-	-	-	-	-
0,01 „.....	++	++	++	+	+	-	-	-	-	-	-
Aqua dest.....	++	++	++	+	±	-	-	-	-	-	-

Beim Agglutinationsversuch durch Rotzbazillen mit Normalpferdeserum ist der Agglutinationstiter des mit 0,05%iger NaCl-Lösung verdünnten serums immer höher als der des mit 0,85%iger NaCl-Lösung verdünnten.

Wenn die Immunkörper im Blut der Pferde, welche mit durch Wärme abgetöteten Rotzbazillen behandelt wurden, aufgetreten sind, ist der Agglutinationstiter des mit 0,85%iger NaCl-Lösung verdünnten Serums höher als der des mit 0,05%iger NaCl-Lösung verdünnten, so dass diese Beziehung vom Immunagglutinationstiter unabhängig ist.

Nun machte ich den Agglutinationsversuch mit dem mittelst 0,85%iger, 0,1%iger und 0,05%iger NaCl-Lösungen oder destilliertes Wasser verdünnten Normalserum und Immunserum von ein und demselben Pferde, welchem nach der einmaligen Entblutung eine bestimmte Menge von der auf 60°C. bei 30 Minuten lang erhitzten Rotzkultur auf Agar intravenös eingespritzt wurde und dessen Blut vom nächsten Tage an in einen brauchbaren Menge aus der Jugular-Vene täglich entnommen wurde, um die Veränderungen der Agglutinationstiter beim Vorschreiten der Immunisierung des Pferdes zu beobachten.

2) Über die Veränderungen des Agglutinationstitors beim Vorschreiten der Immunisierung des Pferdes, welchem durch Wärme abgetötete Rotzbazillen intravenös eingespritzt wurden.

A. Agglutinationsversuch durch Rotzbazillen mit dem mittelst 0,85%iger und 0,05%iger NaCl-Lösungen oder destilliertes Wasser verdünnten Normalserum des Pferdes und mit gleich verdünntem Immunserum desselben, welchem nach einmaliger Entblutung eine Menge abgetöteter Rotzkultur intravenös eingespritzt wurde.

Versuchsmethode: Nachdem ein Agglutinationsversuch unmittelbar vor der Einspritzung ausgeführt worden war, wurde den Versuchspferden, Mizuiwa, Yokofuyu und Shunshu, am 18. Dez. 1916 eine Menge von I/5 Normalöse der auf 60°C. bei 30 Minuten lang erhitzten Rotzkultur auf Agar intravenös eingespritzt und während 3 Wochen vom nächsten Tage an täglich einmalige Entblutung einer brauchbaren Menge aus der Jugular-Vene ausgeführt. So erhaltenes Serum wurde mit 0,05%igen und 0,85%igen NaCl-Lösungen, und destilliertem Wasser verdünnt und zum Versuch verwandt. (Tabelle 4.)

Aus den Resultaten dieses Versuchs ist der Agglutinationstiter des mit

0,85%iger NaCl-Lösung verdünnten Pferdeserum ziemlich höher als der des mit destilliertem Wasser verdünnten,

TABELLE 4.

(Über die Veränderungen des Agglutinationstiter beim Vorschreiten der Immunisierung des Pferdes.)

(1)

Mit dem Serum aus Versuchspferd Yokofuyu.

(Bei der Verdünnung mit 0,85%iger NaCl-Lösung.)

Tage n. Ein-spritz. d. Rotzbaz.	Verdünnung des Serums.		10	20	40	80	160	320	640	1280	2560	Kontrolle.
			++	+	+	+	-	-	-	-	-	-
Normal .....			++	+	+	+	-	-	-	-	-	-
1 .....			++	+	+	+	-	-	-	-	-	-
2 .....			++	+	+	+	-	-	-	-	-	-
3 .....			++	+	+	+	-	-	-	-	-	-
4 .....			++	++	+	+	±	-	-	-	-	-
5 .....			++	++	+	+	+	-	-	-	-	-
6 .....			++	++	+	+	+	-	-	-	-	-
7 .....			++	++	+	+	+	-	-	-	-	-
8 .....			++	##	##	##	##	+	+	+	+	-
9 .....			++	##	##	##	##	+	+	+	+	-
10 .....			++	##	##	##	##	+	+	+	+	-
11 .....			++	##	##	##	##	+	+	+	+	-
12 .....			++	##	##	##	##	+	+	+	+	-
13 .....			++	##	##	##	##	+	+	+	+	-
14 .....			++	##	##	##	##	+	+	+	+	-
15 .....			++	##	##	##	##	+	+	+	+	-
16 .....			++	##	##	##	##	+	+	+	+	-
17 .....			++	##	##	##	##	+	+	+	+	-
18 .....			++	##	##	##	##	+	+	+	+	-
19 .....			++	##	##	##	##	+	+	+	+	-
20 .....			++	##	##	##	##	+	+	+	+	-
21 .....			++	##	##	##	##	+	+	+	+	-

(Bei der Verdünnung mit 0,05%iger NaCl-Lösung.)

Tage n. Einspritz. d. Rotzbaz.	Verdünnung des Serums.	50	100	200	400	800	1600	3200	6400	12800	Kontrolle.
Normal .....	##	##	++	++	+	+	+	+	+	++	-
1 .....	##	##	++	++	+	+	+	+	+	++	-
2 .....	##	##	++	++	+	+	+	+	+	++	-
3 .....	##	##	++	++	+	+	+	+	+	++	-
4 .....	##	##	++	++	+	+	+	+	+	++	-
5 .....	##	##	++	++	+	+	+	+	+	++	-
6 .....	##	##	++	++	+	+	+	+	+	++	-
7 .....	##	##	++	++	+	+	+	+	+	++	-
8 .....	##	##	++	++	+	+	+	+	+	++	-
9 .....	##	##	++	++	+	+	+	+	+	++	-
10 .....	##	##	++	++	+	+	+	+	+	++	-
11 .....	##	##	++	+	-	-	-	-	-	-	-
12 .....	##	##	++	+	-	-	-	-	-	-	-
13 .....	##	##	++	+	-	-	-	-	-	-	-
14 .....	##	##	++	+	-	-	-	-	-	-	-
15 .....	##	##	++	+	-	-	-	-	-	-	-
16 .....	##	##	++	+	-	-	-	-	-	-	-
17 .....	##	##	++	+	-	-	-	-	-	-	-
18 .....	##	##	++	+	-	-	-	-	-	-	-
19 .....	##	##	++	+	-	-	-	-	-	-	-
20 .....	##	##	++	+	-	-	-	-	-	-	-
21 .....	##	##	++	+	-	-	-	-	-	-	-

(Bei der Verdünnung mit destilliertem Wasser.)

Tage n. Einspritz. d. Rotzbaz.	Verdünnung des Serums.	10	20	40	80	160	320	640	Kontr.
Normal .....	##	##	++	+	-	-	-	-	-
1 .....	##	##	##	+	-	-	-	-	-
2 .....	##	##	##	+	-	-	-	-	-
3 .....	##	##	##	+	-	-	-	-	-
4 .....	##	##	##	+	-	-	-	-	-
5 .....	##	##	##	+	-	-	-	-	-

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	Kontr.
6 .....	++	++	++	+	±	-	-	-
7 .....	++	++	++	+	±	-	-	-
8 .....	++	++	++	+	±	-	-	-
9 .....	++	++	++	+	±	-	-	-
10 .....	++	++	++	+	±	-	-	-
11 .....	++	++	++	+	±	-	-	-
12 .....	++	++	++	+	-	-	-	-
13 .....	++	++	++	+	-	-	-	-
14 .....	++	++	++	+	-	-	-	-
15 .....	++	++	++	+	-	-	-	-
16 .....	++	++	++	+	-	-	-	-
17 .....	++	++	++	+	-	-	-	-
18 .....	++	++	++	+	-	-	-	-
19 .....	++	++	++	+	-	-	-	-
20 .....	++	++	++	+	-	-	-	-
21 .....	++	++	++	+	-	-	-	-

( 2 )

Mit dem Serum aus Versuchspferd Shunshu.

(Bei der Verdünnung mit 0,85%iger NaCl-Lösung.)

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	1280	2560	Kontrolle.
Normal .....	+	+	+	+	-	-	-	-	-	-
1 .....	+	+	+	+	-	-	-	-	-	-
2 .....	+	+	+	+	-	-	-	-	-	-
3 .....	+	+	+	+	-	-	-	-	-	-
4 .....	+	++	+	+	±	-	-	-	-	-
5 .....	+	++	+	+	±	-	-	-	-	-
6 .....	+	++	+	+	+	-	-	-	-	-
7 .....	+	++	+	+	+	-	-	-	-	-
8 .....	+	++	++	++	++	-	-	-	-	-
9 .....	+	++	++	++	++	-	-	-	-	-

Tage n. Einspritz. d. Rotzbaz.	Verdünnung des Serums.		10	20	40	80	160	320	640	1280	2560	Kontrolle.
10 .....	#	#	#	#	#	#	#	+	+	+	-	
11 .....	#	#	#	#	#	#	#	+	+	-	-	
12 .....	#	#	#	#	#	#	#	+	+	-	-	
13 .....	#	#	#	#	#	#	#	+	+	-	-	
14 .....	#	#	#	#	#	#	#	+	+	-	-	
15 .....	#	#	#	#	#	#	#	+	+	-	-	
16 .....	#	#	#	#	#	#	#	+	+	-	-	
17 .....	#	#	#	#	#	#	#	+	+	-	-	
18 .....	#	#	#	#	#	#	#	+	+	-	-	
19 .....	#	#	#	#	#	#	#	+	+	-	-	
20 .....	#	#	#	#	#	#	#	+	+	-	-	
21 .....	#	#	#	#	#	#	#	+	+	-	-	

(Bei der Verdünnung mit 0,05%iger NaCl-Lösung.)

Tage n. Einspritz. d. Rotbaz.	Verdünnung des Serums.		50	100	200	400	800	1600	3200	6400	12800	Kontrolle.
Normal .....	#	#	#	#	#	#	#	+	+	+	+	
1 .....	#	#	#	#	#	#	#	+	+	+	+	
2 .....	#	#	#	#	#	#	#	+	+	+	+	
3 .....	#	#	#	#	#	#	#	+	+	+	+	
4 .....	#	#	#	#	#	#	#	+	+	+	+	
5 .....	#	#	#	#	#	#	#	+	+	+	+	
6 .....	#	#	#	#	#	#	#	+	+	+	+	
7 .....	#	#	#	#	#	#	#	+	+	+	+	
8 .....	#	#	#	#	#	#	#	+	+	+	+	
9 .....	#	#	#	#	#	#	#	+	-	-	-	
10 .....	#	#	#	#	#	#	#	+	-	-	-	
11 .....	#	#	#	#	#	#	#	-	-	-	-	
12 .....	#	#	#	#	#	#	#	-	-	-	-	
13 .....	#	#	#	#	#	#	#	-	-	-	-	
14 .....	#	#	#	#	#	#	#	-	-	-	-	
15 .....	#	#	#	#	#	#	#	-	-	-	-	
16 .....	#	#	#	#	#	#	#	-	-	-	-	

Verdünnung des Serum. Tage n. Einspritz. d. Rotzbaz.	50	100	200	400	800	1600	3200	6400	12800	Kontrolle.
	++	++	++	+	-	-	-	-	-	-
17 .....	++	++	++	+	-	-	-	-	-	-
18 .....	++	++	++	+	-	-	-	-	-	-
19 .....	++	++	++	+	-	-	-	-	-	-
20 .....	++	++	++	+	-	-	-	-	-	-
21 .....	++	++	++	+	-	-	-	-	-	-

(Bei der Verdünnung mit destilliertem Wasser.)

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	Kontr.
	++	++	++	+	-	-	-	-
Normal. ....	++	++	++	+	-	-	-	-
1 .....	++	++	++	+	-	-	-	-
2 .....	++	++	++	+	-	-	-	-
3 .....	++	++	++	+	-	-	-	-
4 .....	++	++	++	+	-	-	-	-
5 .....	++	++	++	+	-	-	-	-
6 .....	++	++	++	+	-	-	-	-
7 .....	++	++	++	+	-	-	-	-
8 .....	++	++	++	+	-	-	-	-
9 .....	++	++	++	+	-	-	-	-
10.....	++	++	++	+	-	-	-	-
11.....	++	++	++	+	-	-	-	-
12.....	++	++	++	+	-	-	-	-
13.....	++	++	++	+	-	-	-	-
14.....	++	++	++	+	-	-	-	-
15.....	++	++	++	+	-	-	-	-
16.....	++	++	++	+	-	-	-	-
17.....	++	++	++	+	-	-	-	-
18.....	++	++	++	+	-	-	-	-
19.....	++	++	++	+	-	-	-	-
20.....	++	++	++	+	-	-	-	-
21.....	++	++	++	+	-	-	-	-

( 3 )

Mit dem Serum aus Versuchspferd Mizuwa.

(Bei der Verdünnung mit 0,85%iger NaCl-Lösung.)

Tage n. Ein- spritz. d. Rotzbaz.	Verdünnung des Serum.								Kontrolle.
	10	20	40	80	160	320	640	1280	
Normal.....	++	+	+	±	-	-	-	-	
1 .....	++	+	+	±	-	-	-	-	
2 .....	++	+	+	±	-	-	-	-	
3 .....	++	+	+	±	-	-	-	-	
4 .....	++	+	+	±	-	-	-	-	
5 .....	++	+	+	+	-	-	-	-	
6 .....	++	+	+	+	-	-	-	-	
7 .....	+++	+++	++	+	+	-	-	-	
8 .....	+++	+++	+++	++	+	+	+	+	
9 .....	+++	+++	+++	++	++	+	+	+	
10 .....	+++	+++	+++	++	++	+	+	+	
11 .....	+++	+++	+++	++	++	+	+	+	
12 .....	+++	+++	+++	++	++	+	+	+	
13 .....	+++	+++	+++	++	++	+	+	+	
14 .....	+++	+++	+++	++	++	+	+	+	
15 .....	+++	+++	+++	++	++	+	+	+	
16 .....	+++	+++	+++	++	++	+	+	+	
17 .....	+++	+++	+++	++	++	+	+	+	
18 .....	+++	+++	+++	++	++	+	+	+	
19 .....	+++	+++	+++	++	++	+	+	+	
20 .....	+++	+++	+++	++	++	+	+	+	
21 .....	+++	+++	+++	++	++	+	+	+	

(Bei der Verdünnung mit 0,05%iger NaCl-Lösung.)

Tage n. Ein- spritz. d. Rotzbaz.	Verdünnung des Serums.								Kontrolle.
	50	100	200	400	800	1600	3200	6400	
Normal .....	++	++	++	++	++	+	+	+	
1 .....	++	++	++	++	++	+	+	+	
2 .....	++	++	++	++	++	+	+	+	

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	50	100	200	400	800	1600	3200	6400	12800	Kontrolle.
	++	++	++	++	++	+	+	+	+	—
3 .....	++	++	++	++	++	+	+	+	+	—
4 .....	++	++	++	++	++	+	+	+	+	—
5 .....	++	++	++	++	++	+	+	+	+	—
6 .....	++	++	++	++	++	+	+	+	+	—
7 .....	++	++	++	++	++	+	+	+	+	—
8 .....	++	++	++	++	+	+	+	—	—	—
9 .....	++	++	++	++	+	+	+	—	—	—
10 .....	++	++	++	++	+	+	+	—	—	—
11 .....	++	++	++	++	+	+	+	—	—	—
12 .....	++	++	++	++	+	+	+	—	—	—
13 .....	++	++	++	++	+	+	+	—	—	—
14 .....	++	++	++	++	+	+	+	—	—	—
15 .....	++	++	++	++	+	+	+	—	—	—
16 .....	++	++	++	++	+	+	+	—	—	—
17 .....	++	++	++	++	+	+	+	—	—	—
18 .....	++	++	++	++	+	+	+	+	—	—
19 .....	++	++	++	++	+	+	+	—	—	—
20 .....	++	++	++	++	+	+	+	+	—	—
21 .....	++	++	++	++	+	+	+	—	—	—

(Bei der Verdünnung mit destilliertem Wasser.)

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	Kontr.
	++	++	+	+	—	—	—	—
Normal .....	++	++	+	+	—	—	—	—
1 .....	++	++	+	+	—	—	—	—
2 .....	++	++	+	+	—	—	—	—
3 .....	++	++	+	+	—	—	—	—
4 .....	++	++	+	+	—	—	—	—
5 .....	++	++	+	+	—	—	—	—
6 .....	++	++	+	+	—	—	—	—
7 .....	++	++	+	+	—	—	—	—
8 .....	++	++	++	+	—	—	—	—
9 .....	++	++	++	++	—	—	—	—

Verdünnung des Serums. Tage n, Ein-spritze, d. Rotzbaz.	10	20	40	80	160	320	640	Kontr.
	++	++	++	++	+	±	-	-
10 .....	++	++	++	++	+	±	-	-
11.....	++	++	++	++	+	±	-	-
12.....	++	++	++	++	+	±	-	-
13.....	++	++	++	++	+	±	-	-
14.....	++	++	++	++	+	±	-	-
15.....	++	++	++	++	+	±	-	-
16.....	++	++	++	++	+	-	-	-
17.....	++	++	++	++	+	-	-	-
18.....	++	++	++	++	+	-	-	-
19.....	++	++	++	++	+	-	-	-
20.....	++	++	++	++	+	-	-	-
21.....	++	++	++	++	+	-	-	-

verdünnten, aber der Agglutinationstiter des mit 0,05%iger NaCl-Lösung verdünnten ziemlich niedriger als der des Normalserums beim Auftreten der Immunkörper im Blut; diese Beziehung tritt immer deutlicher beim Vorschreiten der Immunisierung ein, und der Agglutinationstiter des Immunserums ist höher oder niedriger als der des mit 0,85%iger NaCl-Lösung verdünnten.

B. Agglutinationsversuch durch Rotbazillen mit dem mittelst 0,1%iger und 0,03%iger NaCl-Lösungen oder destilliertes Wassers verdünnten Normalserum des Pferdes und mit gleich verdünntem Immunserum desselben, welches mit Rotbazillen eingespritzt wurde.

Versuchsmethode: Nach einem Agglutinationsversuch unmittelbar vor der Entblutung, wurde den Versuchspferden, Yakei, Kanjyo und Tosen, am 11. Jan. 1917 und den Versuchspferden, Seichi, Fukuoka und Sachiyo am 2. Feb. 1917 eine Menge von 1/5 Normalöse der auf 60°C. 30 Minuten lang erhitzen Rotzkultur auf Agar intravenös eingespritzt und während 2 Wochen vom 5ten Tage an nach der Impfung täglich einmalige Entblutung der brauchbaren Menge aus der Jugular-Vene ausgeführt. So erhaltenes Serum wird mit 0,85%igen, 0,01%igen und 0,03%igen NaCl-Lösungen und destilliertem Wasser verdünnt, und zum Versuch verwandt. Die Agglutinationstechnik ist wie bei vorhergehendem Versuch (A) und die Resultate dieses Versuchs zeigt Tabelle 5,

Diesen Versuchsresultaten zufolge ist der Agglutinationstiter durch Rotzbazillen des mit 0,1%iger NaCl-Lösung verdünnten Serums etwas gestiegen und höher als der des mit 0,85%iger NaCl-Lösung verdünnten, und der des mit 0,03%iger NaCl-Lösung verdünnten ist immer niedriger als der des mit 0,85%iger NaCl-Lösung verdünnten beim Auftreten der Immunkörper im Blut.

TABELLE 5.

## Über die Veränderungen des Agglutinationstiter beim Vorschreiten der Immunisierung des Pferdes.

( 1 )

Mit dem Serum aus Versuchspferde Fukuko.

(Bei der Verdünnung mit 0,85%iger NaCl-Lösung.)

(Bei der Verdünnung mit 0,1%iger NaCl-Lösung.)

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	1280	2560	5120	10240	Kontrolle.
	++	++	++	++	++	++	++	++	+	+	+	-
Normal .....	++	++	++	++	++	++	++	++	+	+	+	-
5 .....	++	++	++	++	++	++	++	++	+	+	+	-
6 .....	++	++	++	++	++	++	++	++	+	+	+	-
7 .....	++	++	++	++	++	++	++	++	+	+	+	-
8 .....	++	++	++	++	++	++	++	++	+	+	+	-
9 .....	++	++	++	++	++	++	++	++	+	+	+	-
10 .....	++	++	++	++	++	++	++	++	+	+	+	-
11 .....	++	++	++	++	++	++	++	++	+	+	+	-
12 .....	++	++	++	++	++	++	++	++	+	+	+	-
13 .....	++	++	++	++	++	++	++	++	+	+	+	-
14 .....	++	++	++	++	++	++	++	++	+	+	+	-
15 .....	++	++	++	++	++	++	++	++	+	+	+	-
16 .....	++	++	++	++	++	++	++	++	+	+	+	-
17 .....	++	++	++	++	++	++	++	++	+	+	+	-
18 .....	++	++	++	++	++	++	++	++	+	+	+	-
19 .....	++	++	++	++	++	++	++	++	+	+	+	-
20 .....	++	++	++	++	++	++	++	++	+	+	+	-

(Bei der Verdünnung mit 0,03%iger NaCl-Lösung.)

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	1280	2560	Kontrolle.
	++	++	++	++	+	+	+	+	+	-
Normal .....	++	++	++	++	+	+	+	+	+	-
5 .....	++	++	++	++	+	+	+	+	+	-
6 .....	++	++	++	++	+	+	+	+	+	-
7 .....	++	++	++	++	+	+	+	+	+	-
8 .....	++	++	++	++	+	+	+	+	+	-
9 .....	++	++	++	++	+	+	+	+	+	-
10 .....	++	++	++	++	+	+	+	+	+	-
11 .....	++	++	++	++	+	+	+	+	+	-
12 .....	++	++	++	++	+	+	+	+	+	-
13 .....	++	++	++	++	+	+	+	+	+	-
14 .....	++	++	++	++	+	+	+	+	+	-

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	1280	2560	Kontrolle.
	++	++	++	+	+	-	-	-	-	
15 .....	++	++	++	+	+	-	-	-	-	-
16 .....	++	++	++	+	+	-	-	-	-	-
17 .....	++	++	++	+	+	-	-	-	-	-
18 .....	++	++	++	+	+	-	-	-	-	-
19 .....	++	++	++	+	+	-	-	-	-	-
20 .....	++	++	++	+	+	-	-	-	-	-

(Bei der Verdünnung mit destilliertem Wasser.)

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	Kontr.
	++	++	++	+	±	-	-	-
Normal .....	++	++	++	+	±	-	-	-
5 .....	++	++	++	+	±	-	-	-
6 .....	++	++	++	+	±	-	-	-
7 .....	++	++	++	+	±	-	-	-
8 .....	++	++	++	+	±	-	-	-
9 .....	++	++	++	+	+	-	-	-
10 .....	++	++	++	+	+	-	-	-
11 .....	++	++	++	+	+	-	-	-
12 .....	++	++	++	+	+	-	-	-
13 .....	++	++	++	+	+	-	-	-
14 .....	++	++	++	+	+	-	-	-
15 .....	++	++	++	+	+	-	-	-
16 .....	++	++	++	+	+	-	-	-
17 .....	++	++	++	+	+	-	-	-
18 .....	++	++	++	+	+	-	-	-
19 .....	++	++	++	+	+	-	-	-
20 .....	++	++	++	+	+	-	-	-

( 2 )

Mit dem Serum aus Versuchspferde Kanjyo.

(Bei der Verdünnung mit 0,85%iger NaCl-Lösung.)

(Bei der Verdünnung mit 0,1%iger NaCl-Lösung.)

Verdünnung des Serums. Tage n. Ein- spritz. d. Rotzbaz.	10	20	40	80	160	320	640	1280	2560	5120	10240	Kontrolle.
	++	++	++	++	++	++	++	++	++	++	++	—
11.....	++	++	++	++	++	++	++	++	++	++	++	—
12.....	++	++	++	++	++	++	++	++	++	++	++	—
13.....	++	++	++	++	++	++	++	++	++	++	++	—
14.....	++	++	++	++	++	++	++	++	++	++	++	—
15.....	++	++	++	++	++	++	++	++	++	++	++	—
16.....	++	++	++	++	++	++	++	++	++	++	++	—
17.....	++	++	++	++	++	++	++	++	++	++	++	—
18.....	++	++	++	++	++	++	++	++	++	++	++	—
19.....	++	++	++	++	++	++	++	++	++	++	++	—
20.....	++	++	++	++	++	++	++	++	++	++	++	—

(Bei der Verdünnung mit 0,03%iger NaCl-Lösung.)

Verdünnung des Serums. Tage n. Ein- spritz. d. Rotzbaz.	10	20	40	80	160	320	640	1280	2560	Kontrolle.
	++	++	++	++	++	++	++	++	++	—
Normal.....	++	++	++	++	++	++	++	++	++	—
5 .....	++	++	++	++	++	+	+	+	+	—
6 .....	++	++	++	++	++	+	+	+	+	—
7 .....	++	++	++	++	++	+	+	+	+	—
8 .....	++	++	++	++	++	+	+	+	+	—
9 .....	++	++	++	++	++	+	+	+	+	—
10 .....	++	++	++	++	++	+	+	+	+	—
11 .....	++	++	++	++	++	+	+	+	+	—
12 .....	++	++	++	++	++	+	+	+	+	—
13 .....	++	++	++	++	++	+	+	+	+	—
14 .....	++	++	++	++	++	+	+	+	+	—
15 .....	++	++	++	++	++	+	+	+	+	—
16 .....	++	++	++	++	++	+	+	+	+	—
17 .....	++	++	++	++	++	+	+	+	+	—
18 .....	++	++	++	++	++	+	+	+	+	—
19 .....	++	++	++	++	++	+	+	+	+	—
20 .....	++	++	++	++	++	+	+	+	+	—

(Bei der Verdünnung mit destilliertem Wasser.)

Verdünnung des Serums. Tage n. Ein- spritzen d. Rotzbaz.	10	20	40	80	160	320	640	Kontr.
	++	++	++	+	±	-	-	-
Normal .....	++	++	++	+	±	-	-	-
5 .....	++	++	++	+	±	-	-	-
6 .....	++	++	++	+	±	-	-	-
7 .....	++	++	++	+	±	-	-	-
8 .....	++	++	++	+	±	-	-	-
9 .....	++	++	++	+	+	-	-	-
10.....	++	++	++	++	+	-	-	-
11.....	++	++	++	++	+	-	-	-
12.....	++	++	++	++	+	-	-	-
13.....	++	++	++	++	+	-	-	-
14.....	++	++	++	++	+	-	-	-
15.....	++	++	++	++	+	-	-	-
16.....	++	++	++	++	+	-	-	-
17.....	++	++	++	++	+	-	-	-
18.....	++	++	++	++	+	-	-	-
19.....	++	++	++	++	+	-	-	-
20.....	++	++	++	++	+	-	-	-

( 3 )

Mit dem Serum aus Versuchspferd Yakei.

(Bei der Verdünnung mit 0,85%iger NaCl-Lösung.)

Tage n. Ein-spritz. d. Rotzbaz.	Verdünnung des Serums.						Kontrolle.
	10	20	40	80	160	320	
11.....	#	#	#	#	#	+	
12 .....	#	#	#	#	#	+	20
13.....	#	#	#	#	#	+	
14 .....	#	#	#	#	#	+	40
15.....	#	#	#	#	#	+	
16 .....	#	#	#	#	#	+	
17.....	#	#	#	#	#	+	
18.....	#	#	#	#	#	+	
19.....	#	#	#	#	#	+	
20.....	#	#	#	#	#	+	

(Bei der Verdünnung mit 0,1%iger NaCl-Lösung.)

Tage n. Ein-spritz. d. Rotzbaz.	Verdünnung des Serums.						Kontrolle.
	10	20	40	80	160	320	
Normal .....	#	#	#	#	#	#	
5 .....	#	#	#	#	#	#	
6 .....	#	#	#	#	#	#	
7 .....	#	#	#	#	#	#	
8 .....	#	#	#	#	#	#	
9 .....	#	#	#	#	#	#	
10.....	#	#	#	#	#	#	
11.....	#	#	#	#	#	#	
12.....	#	#	#	#	#	#	
13.....	#	#	#	#	#	#	
14.....	#	#	#	#	#	#	
15.....	#	#	#	#	#	#	
16.....	#	#	#	#	#	#	
17.....	#	#	#	#	#	#	
18.....	#	#	#	#	#	#	
19.....	#	#	#	#	#	#	
20.....	#	#	#	#	#	#	

(Bei der Verdünnung mit 0,03%iger NaCl-Lösung.)

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	1280	2560	Kontrolle.
Normal.....	++	++	++	++	++	+	+	+	-	-
5 .....	++	++	++	++	+	+	+	-	-	-
6 .....	++	++	++	++	+	+	+	-	-	-
7 .....	++	++	++	++	+	+	+	-	-	-
8 .....	++	++	++	++	+	+	+	-	-	-
9 .....	++	++	++	++	+	+	+	-	-	-
10 .....	++	++	++	++	+	+	+	-	-	-
11 .....	++	++	++	++	+	+	+	-	-	-
12 .....	++	++	++	++	+	+	+	-	-	-
13 .....	++	++	++	++	+	+	+	-	-	-
14 .....	++	++	++	++	+	+	+	-	-	-
15 .....	++	++	++	++	+	+	+	-	-	-
16 .....	++	++	++	++	+	+	+	-	-	-
17 .....	++	++	++	++	+	+	+	-	-	-
18 .....	++	++	++	++	+	+	+	-	-	-
19 .....	++	++	++	++	+	+	+	-	-	-
20 .....	++	++	++	++	+	+	+	-	-	-

(Bei der Verdünnung mit destilliertem Wasser.)

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	Kontr.
Normal .....	++	++	++	+-	±	-	-	-
5 .....	++	++	++	+	±	-	-	-
6 .....	++	++	++	+	±	-	-	-
7 .....	++	++	++	+	±	-	-	-
8 .....	++	++	++	+	±	-	-	-
9 .....	++	++	++	++	+	-	-	-
10 .....	++	++	++	++	+	-	-	-
11 .....	++	++	++	++	+	-	-	-
12 .....	++	++	++	++	+	-	-	-
13 .....	++	++	++	++	+	-	-	-
14 .....	++	++	++	++	+	-	-	-



(Bei der Verdünnung mit 0,1%iger NaCl-Lösung.)

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	1280	2560	5120	10240	Kontrolle.
	++	++	++	++	++	++	++	++	++	++	++	—
Normal .....	++	++	++	++	++	++	++	++	++	++	++	—
5 .....	++	++	++	++	++	++	++	++	++	++	++	—
6 .....	++	++	++	++	++	++	++	++	++	++	++	—
7 .....	++	++	++	++	++	++	++	++	++	++	++	—
8 .....	++	++	++	++	++	++	++	++	++	++	++	—
9 .....	++	++	++	++	++	++	++	++	++	++	++	—
10 .....	++	++	++	++	++	++	++	++	++	++	++	—
11 .....	++	++	++	++	++	++	++	++	++	++	++	—
12 .....	++	++	++	++	++	++	++	++	++	++	++	—
13 .....	++	++	++	++	++	++	++	++	++	++	++	—
14 .....	++	++	++	++	++	++	++	++	++	++	++	—
15 .....	++	++	++	++	++	++	++	++	++	++	++	—
16 .....	++	++	++	++	++	++	++	++	++	++	++	—
17 .....	++	++	++	++	++	++	++	++	++	++	++	—
18 .....	++	++	++	++	++	++	++	++	++	++	++	—
19 .....	++	++	++	++	++	++	++	++	++	++	++	—
20 .....	++	++	++	++	++	++	++	++	++	++	++	—

(Bei der Verdünnung mit 0,03%iger NaCl-Lösung.)

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	1280	Kontrolle.
	++	++	++	++	++	+	+	+	—
Normal .....	++	++	++	++	++	+	+	+	—
5 .....	++	++	++	++	++	+	+	+	—
6 .....	++	++	++	++	++	+	+	+	—
7 .....	++	++	++	++	++	+	+	+	—
8 .....	++	++	++	++	++	+	+	+	—
9 .....	++	++	++	++	++	+	+	+	—
10 .....	++	++	++	++	++	+	+	+	—
11 .....	++	++	++	++	++	+	+	+	—
12 .....	++	++	++	++	++	+	+	+	—
13 .....	++	++	++	++	++	+	+	+	—
14 .....	++	++	++	++	++	+	+	+	—

Verdünnung des Serums. Tage n. Ein- spritz. d. Rotzbaz.	10	20	40	80	160	320	640	1280	Kontrolle.
	++	++	++	++	+	+	±	-	
15 .....	++	++	++	++	+	+	±	-	-
16 .....	++	++	++	++	+	+	±	-	-
17 .....	++	++	++	++	+	+	±	-	-
18 .....	++	++	++	++	+	+	±	-	-
19 ..	++	++	++	++	+	+	±	-	-
20 .....	++	++	++	++	+	+	±	-	-

(Bei der Verdünnung mit destilliertem Wasser.)

Verdünnung des Serums. Tage n. Ein- spritz. d. Rotzbaz.	10	20	40	80	160	320	640	Konfr.
	++	++	++	+	±	-	-	-
Normal .....	++	++	++	+	±	-	-	-
5 .....	++	++	++	+	±	-	-	-
6 .....	++	++	++	+	±	-	-	-
7 .....	++	++	++	+	±	-	-	-
8 .....	++	++	++	+	±	-	-	-
9 .....	++	++	++	+	+	-	-	-
10.....	++	++	++	+	+	-	-	-
11.....	++	++	++	+	+	-	-	-
12.....	++	++	++	+	+	-	-	-
13.....	++	++	++	+	+	-	-	-
14.....	++	++	++	+	+	-	-	-
15.....	++	++	++	+	+	-	-	-
16.....	++	++	++	+	+	-	-	-
17.....	++	++	++	+	+	-	-	-
18.....	++	++	++	+	+	-	-	-
19.....	++	++	++	+	+	-	-	-
20 .....	++	++	++	+	+	-	-	-

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## Mit dem Serum aus Versuchspferde Tosen.

(Bei der Verdünnung mit 0,85%iger NaCl-Lösung.)

	Verdünnung des Serums.			
Tage n. Einspritz. d. Rotzbaz.	10	20	40	80
Normal .....	+	+		
5 .....	#	#	+	+
6 .....	#	#	#	+
7 .....	#	#	#	+
8 .....	#	#	#	+
9 .....	#	#	#	+
10 .....	#	#	#	+
11 .....	#	#	#	+
12 .....	#	#	#	+
13 .....	#	#	#	+
14 .....	#	#	#	+
15 .....	#	#	#	+
16 .....	#	#	#	+
17 .....	#	#	#	+
18 .....	#	#	#	+
19 .....	#	#	#	+
20 .....	#	#	#	+
				Kontrolle.

(Bei der Verdünnung mit 0,1%iger NaCl-Lösung.)

Tage n. Einspritz. d. Rotzbaz.	Verdünnung des Serums.								Kontrolle.
	10	20	40	80	160	320	640	1280	
11.....	#	#	#	#	#	#	#	#	+
12.....	#	#	#	#	#	#	#	#	+
13.....	#	#	#	#	#	#	#	#	+
14.....	#	#	#	#	#	#	#	#	+
15.....	#	#	#	#	#	#	#	#	+
16.....	#	#	#	#	#	#	#	#	+
17.....	#	#	#	#	#	#	#	#	+
18.....	#	#	#	#	#	#	#	#	+
19.....	#	#	#	#	#	#	#	#	+
20.....	#	#	#	#	#	#	#	#	-

(Bei der Verdünnung mit 0,03%iger NaCl-Lösung.)

Tage n. Einspritz. d. Rotzbaz.	Verdünnung des Serums.								Kontrolle.
	10	20	40	80	160	320	640	1280	
Normal.....	#	#	#	#	#	#	+	+	-
5.....	#	#	#	#	#	+	+	+	-
6.....	#	#	#	#	#	+	+	+	-
7.....	#	#	#	#	#	+	+	+	-
8.....	#	#	#	#	#	+	+	+	-
9.....	#	#	#	#	#	+	+	+	-
10.....	#	#	#	#	#	+	+	+	-
11.....	#	#	#	#	#	+	+	+	-
12.....	#	#	#	#	#	+	+	+	-
13.....	#	#	#	#	#	+	+	+	-
14.....	#	#	#	#	#	+	+	+	-
15.....	#	#	#	#	#	+	+	+	-
16.....	#	#	#	#	#	+	+	+	-
17.....	#	#	#	#	#	+	+	+	-
18.....	#	#	#	#	#	+	+	+	-
19.....	#	#	#	#	#	+	+	+	-
20.....	#	#	#	#	#	+	+	+	-

(Bei der Verdünnung mit destilliertem Wasser.)

Tage n. Einspritz. d. Rotzbaz.	Verdünnung des Serums.	10	20	40	80	160	320	640	Kontr.
Normal .....	+++	+++	++	+	±	-	-	-	-
5 .....	+++	+++	++	+	±	-	-	-	-
6 .....	+++	+++	++	+	±	-	-	-	-
7 .....	+++	+++	++	+	±	-	-	-	-
8 .....	+++	+++	++	+	±	-	-	-	-
9 .....	+++	+++	++	+	+	-	-	-	-
10.....	+++	+++	++	+	+	-	-	-	-
11.....	+++	+++	++	+	+	-	-	-	-
12.....	+++	+++	++	+	+	-	-	-	-
13.....	+++	+++	++	+	+	-	-	-	-
14.....	+++	+++	++	+	+	-	-	-	-
15.....	+++	+++	++	+	+	-	-	-	-
16.....	+++	+++	++	+	+	-	-	-	-
17.....	+++	+++	++	+	+	-	-	-	-
18.....	+++	+++	++	+	+	-	-	-	-
19.....	+++	+++	++	+	+	-	-	-	-
20.....	+++	+++	++	+	+	-	-	-	-

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Mit dem Serum aus Versuchspferde Seichi.

(Bei der Verdünnung mit 0,85%iger NaCl-Lösung.)

Tage n. Ein- spritz. d. Rotzbaz.	Verdünnung des Serums.						Kontrolle.
	10	20	40	80	160	320	
11.....	+++	++	+	+	+	+	
12 .....	+++	++	+	+	+	+	
13 .....	+++	++	+	+	+	+	
14 .....	+++	++	+	+	+	+	
15 .....	+++	++	+	+	+	+	
16 .....	+++	++	+	+	+	+	
17 .....	+++	++	+	+	+	+	
18 .....	+++	++	+	+	+	+	
19 .....	+++	++	+	+	+	+	
20.....	+++	++	+	+	+	+	

(Bei der Verdünnung mit 0,1%iger NaCl-Lösung.)

Tage n. Ein- spritz. d. Rotzbaz.	Verdünnung des Serums.						Kontrolle.
	10	20	40	80	160	320	
Normal .....	+++	++	++	++	++	++	
5 .....	++	++	++	++	++	++	
6 .....	++	++	++	++	++	++	
7 .....	++	++	++	++	++	++	
8 .....	++	++	++	++	++	++	
9 .....	++	++	++	++	++	++	
10.....	++	++	++	++	++	++	
11.....	++	++	++	++	++	++	
12.....	++	++	++	++	++	++	
13.....	++	++	++	++	++	++	
14.....	++	++	++	++	++	++	
15.....	++	++	++	++	++	++	
16.....	++	++	++	++	++	++	
17.....	++	++	++	++	++	++	
18.....	++	++	++	++	++	++	
19.....	++	++	++	++	++	++	
20.....	++	++	++	++	++	++	

(Bei der Verdünnung mit 0,03%iger NaCl-Lösung.)

Tage n. Ein- spritz. d. Rotzbaz.	Verdünnung des Serums.								Kontrolle.
	10	20	40	80	160	320	640	1280	
Normal.....	++	++	++	++	+	+	+	+	-
5.....	++	++	++	++	+	-	-	-	-
6.....	++	++	++	++	+	-	-	-	-
7.....	++	++	++	+	±	-	-	-	-
8.....	++	++	++	+	±	-	-	-	-
9.....	++	++	++	+	±	-	-	-	-
10.....	++	++	++	+	±	-	-	-	-
11.....	++	++	++	+	±	-	-	-	-
12.....	++	++	++	+	±	-	-	-	-
13.....	++	++	++	+	±	-	-	-	-
14.....	++	++	++	+	+	-	-	-	-
15.....	++	++	++	+	+	-	-	-	-
16.....	++	++	++	+	+	-	-	-	-
17.....	++	++	++	+	+	-	-	-	-
18.....	++	++	++	+	+	-	-	-	-
19.....	++	++	++	+	+	-	-	-	-
20.....	++	++	++	+	+	-	-	-	-

(Bei der Verdünnung mit destilliertem Wasser.)

Tage n. Ein- spritz. d. Rotzbaz.	Verdünnung des Serums.								Kontr.
	10	20	40	80	160	320	640	1280	
Normal.....	++	++	++	+	-	-	-	-	-
5.....	++	++	++	+	-	-	-	-	-
6.....	++	++	++	+	-	-	-	-	-
7.....	++	++	++	+	-	-	-	-	-
8.....	++	++	++	+	-	-	-	-	-
9.....	++	++	++	+	-	-	-	-	-
10.....	++	++	++	+	-	-	-	-	-
11.....	++	++	++	+	-	-	-	-	-
12.....	++	++	++	+	+	-	-	-	-
13.....	++	++	++	+	+	-	-	-	-

Verdünnung des Serums. Tage n. Einspritz. d. Rotzbaz.	10	20	40	80	160	320	640	Kontr.
	++	++	++	+	-	-	-	-
14 .....	++	++	++	+	-	-	-	-
15 .....	++	++	++	+	-	-	-	-
16 .....	++	++	++	+	-	-	-	-
17 ..	++	++	++	+	-	-	-	-
18 .....	++	++	++	+	-	-	-	-
19 .....	++	++	++	+	-	-	-	-
20 .....	++	++	++	+	-	-	-	-

Am 18. und 23. Jan. 1917 wurde die Entblutung aus der Jugular-Vene der Pferde, welche diesem Institut angehören, vorgenommen und dann wurde das ausgeschiedene Serum unter Verdünnung mit 0,85%igen und 0,03%igen NaCl-Lösungen zu dem Versuch mit Rotzbazillen verwandt.

Die Agglutinationstechnik war dieselbe wie beim vorhergehenden Versuch und die Resultate dieses Versuchs zeigt Tabelle 6.

TABELLE 6.

Agglutinationsversuch durch Rotzbazillen mit normalem Pferdeserum.

Nr. 1.

Verdünnung des Serums. % der NaCl-Lös. zur Verdünnung des Serums.	10	20	40	80	160	320	640	1280	2560	5120	Kontrolle.
	++	++	++	++	+	+	+	-	-	-	-
0,85 % .....	++	++	++	++	+	+	+	-	-	-	-
0,03 % .....	++	++	++	++	++	+	+	-	+	-	-
Aqua dest. .....	++	++	++	++	+	-	-	-	-	-	-

## Nr. 2.

Verdünnung des Serums. % der NaCl-Lös. zur Verdünnung des Serums.											Kontrolle.
	10	20	40	80	160	320	640	1280	2560	5120	
0,85 % .....	#	#	+	+	+	+	+	+	-	-	-
0,03 % .....	#	#	+	+	+	+	+	+	+	-	-
Aqua dest. .....	#	#	+	+	+	+	-	-	-	-	-

Diesen Versuchsresultaten zufolge kann man den Agglutinationstiter durch Rotzbazillen des mit 0,03%iger NaCl-Lösung verdünnten Normalpferde-serums immer höher annehmen als den des mit 0,85%iger verdünnten, obgleich der Normalagglutinationstiter ziemlich hoch ist.

Bei der Ausführung der oben erwähnten Versuche habe ich die Test-Flüssigkeiten nach SCHÜTZ und MIESSNER (6), JOEST (7), SUSTMANN (8), WAY (9) und FICKER (10), welche lang konserviert werden sollen, verwandt und die Kontrollversuche gegen lebende Test-Flüssigkeit mehrmals wiederholt, und keinen Unterschied zwischen lebender und abgetöteter Test-Flüssigkeit beobachtet.

Aus oben beschriebenen Versuchsresultaten glaube ich dargetan zu haben, dass die durch hohen Normalagglutinationstiter vorgekommenden Fehldiagnosen beim Agglutinationsversuch rotziger Pferde vermieden werden können, wenn man 2 Reihen-Aggelutinationsversuche mit dem mit 0,85%igen und 0,03%igen NaCl-Lösungen verdünnten Pferdeserum ausführt. Das Pferd ist gesund, wenn der Agglutinationstiter des mit 0,03%iger NaCl-Lösung verdünnten Serums höher ist als der des mit 0,85%iger NaCl-Lösung verdünnten, anderseits ist das Pferd infiziert, wenn der Agglutinationstiter des mit 0,03%iger NaCl-Lösung verdünnten Serums gleich oder niedriger ist als der des mit 0,85%iger NaCl-Lösung verdünnten. Diese Resultate sind wenigstens bei künstlich immunisierten Pferden zu erhalten.

Zur Bestätigung der praktischen Anwendbarkeit dieser Methode muss man das Serum des natürlich infizierten Pferdes zu dem Versuch brauchen. Glücklicherweise hatte ich Gelegenheit, das Serum eines natürlich infizierten rotzigen Pferdes aus der Mandschurei zu erhalten und dasselbe zu meinem Versuch zu verwenden.

C. 2 Reihen-Agglutinationsversuche durch Rotzbazillen mit dem Serum eines natürlich infizierten rotzigen Pferdes.

Versuchsmethode: Um zu bestätigen, ob das gesandte Serum von einem rotzigen Pferde stammt oder nicht, wird Komplementbindungs methode angestellt. Dann wird eine vollständige Ablenkung des Komplements durch das Serum in der Menge von 0,2 cem. hervorgerufen, eine unvollständige Ablenkung dagegen durch 0,1 cem. deselben.

Aus diesem Resultat ist das Pferd als rotzkrank anzusehen, ohne Rücksicht auf die Höhe des Agglutinationstitors, nach den veterinarpolizeilichen Vorschriften für Preussen und nach den klinischen Erscheinungen und dem positiven Ausfall der Ophthalmoreaktion mit Mallein, über welche Herr Kobayashi, Chef des Veterinärkorps der 17 Division berichtet.

Die Versuchsmethode ist die gleiche wie bei vorhergehenden Versuchen und die Resultate Tabelle 7 zeigt.

TABELLE 7.

2 Reihen-Agglutinationsversuche durch Rotzbazillen mit natürlich infiziertem rotzigen Pferdeserum.

Verdünnung des Serums. % der NaCl-Lös. zur Verdünnung des Serums.	Verdünnung des Serums.								Kontrolle.
	10	20	40	80	160	320	640	1280	
0,85 % .....	++	++	++	+	+	+	+	-	-
0,03 % .....	++	++	++	+	+	+	-	-	-

Der Agglutinationstiter des mit 0,85%iger NaCl-Lösung verdünnten rotzkranken Serums durch Rotzbazillen ist immer höher als der des mit 0,03%iger verdünnten, wie beim künstlichen Immunserum.

Aus oben erwähnten Resultaten glaube ich behaupten zu dürfen, dass der Agglutinationsversuch, welcher infolge hohen Normalagglutinationstitors des Normalpferdeserums bisher unbrauchbar war, bei der Anwendung zur Serodiagnostik des Rotzes wertvoll ist.

### Zusammenfassungen.

1. Beim Agglutinationsversuch durch Rotzbazillen mit Rotz-Immunkörperdeserum ist der Agglutinationstiter desselben am höchsten bei der Verdünnung mit 0,1%iger NaCl-Lösung.
2. Beim Agglutinationsversuch durch Rotzbazillen ist der Agglutinationstiter des mit 0,85%iger NaCl-Lösung verdünnten Serums etwas höher als der des mit destilliertem Wasser verdünnten beim Auftreten der Immunkörper im Blut und diese Beziehung tritt immer deutlicher beim Vorschreiten der Immunisierung auf. Aber der Agglutinationstiter des mit 0,05%iger NaCl-Lösung verdünnten Serums ist immer niedriger als der des Normalserums beim Auftreten der Immunkörper und der Agglutinationstiter desselben Serums ist höher oder niedriger als der des Normalserums beim Auftreten der Immunkörper und der Agglutinationstiter desselben Serums ist höher oder niedriger als der des mit 0,85%iger NaCl-Lösung verdünnten, so dass sich keine Einheitlichkeit zeigt.
3. Beim Agglutinationsversuch durch Rotzbazillen ist der Agglutinationstiter des mit 0,1%iger NaCl-Lösung verdünnten Serums etwas gestiegen und höher als der des mit 0,85%iger NaCl-Lösung verdünnten, und der des mit 0,03%iger NaCl-Lösung verdünnten ist immer niedriger als der des mit 0,85%iger NaCl-Lösung verdünnten Serums, beim Auftreten der Immunkörper im Blut.
4. Es zeigt sich kein deutlicher Unterschied zwischen lebenden und konservierbaren Test-Flüssigkeiten beim Agglutinationsversuch durch Rotzbazillen.
5. Sollen keine Fehler bei der Rotzdiagnose des Pferdes vorkommen, so muss man 2 Reihen-Agglutinationsversuche mit den mit 0,85%igen und 0,03%igen NaCl-Lösungen verdünnten Pferdeserum ausführen.  
Das Pferd ist dann gesund, wenn der Agglutinationstiter des mit 0,03%iger NaCl-Lösung verdünnten Serums höher ist als der des mit 0,85%iger NaCl-Lösung verdünnten; demgegenüber ist das Pferd infiziert, wenn der Agglutinationstiter des mit 0,03%iger NaCl-Lösung verdünnten Serums gleich oder niedriger ist als der des mit 0,85%iger NaCl-Lösung verdünnten.

Zum Schlusse kann ich mir nicht versagen, meinen hochverehrten Lehrern, Herrn Prof. Dr. C. YOKOTE, Herrn Prof. Dr. H. HAYASHI und Herrn Assist.-Prof. Dr. M. TAKENOUCHE, für ihre ständige Leitung und Unterstützung bei der Ausführung dieser Arbeit meinen ergebensten Dank auszusprechen.

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# On the toxic Constituents in the Bark of *Robinia pseudacacia L.*

(REPORT I.)

By

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## Introduction.

In the year 1890, B. POWER and J. CAMBIER<sup>1</sup> found a toxic albumose in the aqueous extract of the bark of *Robinia pseudacacia L.* Afterwards this toxic constituent was termed 'robin' by FRIEDRICK B. POWER<sup>2</sup> (1901). According to him, robin, a precipitate from the aqueous extract with acid, has the properties of nucleoprotein, containing a large quantity of iron in its ash. It coagulates by heating from its water solution, complete coagulation taking place at 70—80°C. whereby it loses its toxic action. But, when prepared from the aqueous extract with strong alcohol, it is considered to have the nature of a ferment, as it decomposes amygdalin. By the action of robin, the blood corpuscles of certain animals agglutinate as is the case with ricin, crotin and abrin, and milk caseinogen coagulates as with chymosin. When decomposed, an alkaloid-like substance is obtained.

Treating the aqueous extract with hydrochloric acid, POWER found a crystalline, a noncrystalline substance (named syringenin) and a laevorotatory sugar. This crystalline substance was found to be syringaic acid on account of its constitution and melting point, so that he supposed the existence of a glucoside 'syringin' in the bark, which decomposes by hydrolysis into syringaic acid, syringenin and sugar. But he said nothing about the toxicity of this glucoside.

1. Jahresberichte der Pharmacie. 1890.

2. " " " 1901.

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In the flower of *Robinia pseudacacia* a glucoside named robinin<sup>1</sup> ( $C_{25}H_{30}O_{16}$  +  $5\frac{1}{2}H_2O$ ) was found. It is a fine, yellowish, needle-like crystal, melting at 195°C. and dissolves a little in cold, but easily in boiling water as well as in boiling alcohol, with yellow colour. When boiled with dilute acids, it decomposes into isouleit and quercetin.

According to ZAPEL,<sup>2</sup> the symptoms of intoxication by the bark are very complex. Horses show constipation, staring eyes, acceleration of pulsation and respiration and hyperæmy of visible mucous membranes. At the first stage of the intoxication, the hind part of the body becomes weak, but later paralytic. In the post mortem examination, the liquefaction of intestinal content, hyperæmy of intestinal mucous membranes, remarkable lung oedema and incomplete coagulation of blood are observed. By the hypodermic injection of robin, rabbits suffer from nephritis with thoracic and abdominal exudation, and protein, cylinder and blood in urine are proved. In 1916, an experimental study on Robinia bark with horses was given in the "Rikugun-Jûi-Dampô" (Journal of the Military Veterinary Corps in Japan) No. 81. As result, the following symptoms are recorded: (1) dyspnoea, (2) colic, (3) fatigue, (4) hyperæmy of visible mucous membranes, (5) diarrhoea, (6) pupillary dilatation and (7) secretion of sweat. And post mortem examination shows (1) haemorrhagia cerebri, (2) hyperæmia et emphysema pulmonum, (3) dilatatio cordis et endocarditis chronica, (4) enteritis catarrhalis and (5) nephritis parenchymatosa.

As the locust tree is much cultivated in Japan and her colonies, we considered it to be very important for veterinary hygiene to investigate the toxic properties of its bark. After carrying out a brief experiment, we were able to isolate a new toxic constituent belonging to the glucosides. It may not be very pure as we have not yet succeeded in crystallising it, but for convenience of explanation, we venture to propose a name for it, viz. 'Robitin,' and in the following we give the results so far obtained from the study of it.

1. SCHMIDT, Pharmazeutische Chemie II.
2. KOBERT, Lehrbuch der Intoxikation II, 1906.

### Preliminary experiment.

To ascertain what symptoms can be produced by the fresh bark, the fresh material, sent from the Hokkaidô district, was chopped fine and given, mixed with bran of wheat, to two horses, whose usual evening and morning rations previous to the experiment were discontinued. The rise of temperature after the administration of the bark was 2°C. in one horse and 1°C. in the other, the other symptoms being almost the same in both.

Example. June 13th, 1916

Name of horse Ômi. 9 years old. Body weight 450 kilos.

Time	T.	R.	P.	Symptoms
12°-13'	38.6	14	60	Normal.
1°-0'	—	—	—	200 gms. of the bark given.
40'	—	—	—	All eaten up.
2°-15'	—	—	—	Defæcation (faeces 50 boli, normal consistence).
20'	—	—	—	„ (faeces 12 boli, normal consistence).
40'	—	—	—	„ (faeces 25 boli, a little soft, mucus adhering).
42'	—	—	—	„ (faeces 20 boli, slightly soft), salivation and nasal discharge.
52'	—	—	—	Defæcation (faeces 25 boli, like cattle faeces), beginning to paw, a little uneasy and excited.
3°-0'	39.3	34	64	Sometimes pawing, peristalsis becomes weak, conjunctival hyperæmy, sacral reflex slightly dull.
30'	—	—	—	Hind part of body becomes a little weak.
4°-0'	39.3	18	60	Defæcation (about 2 litres, muddy, having unpleasant smell, mixed with mucus), pulse weak, abdominal type of respiration, peristalsis very weak, indifferent to flies gathering on the skin, legs feel cold.
5°-0'	39.5	20	60	Peristalsis a little strong at right side, but weak at left.
15'	—	—	—	Defæcation (1 litre, like cattle faeces), a metallic sound in peristalsis.
6°-0'	39.5	14	66	Defæcation (0.5 litres, very soft and mixed with much pseudomembrane), salivation, strong hyperæmia of visible mucous membranes, tearing.
7°-0'	39.5	14	60	Peristalsis being excited, penis lowers.
35'	—	—	—	Defæcation (1 litre, muddy, mixed with many undigested grains of barley), ramblings to be heard.

Time	T.	R.	P.	Symptoms
8°-0'	40.0	12	78	Coughing.
35'	—	—	—	Tearing stops.
9°-0'	39.8	15	84	—
25'	—	—	—	Defæcation (faeces 3 boli, soft).
10°-0'	39.8	26	72	Symptoms of dyspnoea.
11°-30'	39.8	32	72	Defæcation (faeces 13 boli, muddy), strong dyspnoea, sometimes accompanied with nausea.
12°-25'	39.8	54	84	Strong dyspnoea, wind sound of nose to be heard distinctly, neck stretching forward with nose reaching to the ground, strong nausea.
40'	—	—	—	Defæcation (faeces 13 boli, muddy, smelling badly, mixed with pseudomembranes).
1°-10'	—	—	—	Watery diarrhoea (2 litres).
40'	39.4	51	75	Defæcation (faeces 15 boli, muddy), nausea frequent, great difficulty in breathing, movement of the abdominal walls to be seen markedly, nasal sound being very loud, moistness of mucosa increased, frequent smacking with the tongue, very disturbed, and presenting a pitiful appearance.
2°-0'	—	—	—	Taking hay, but mastication listless, peristalsis to be heard only on the left, indistinctly.
4°-30'	38.3	30	66	Urination (urine 1 litre, very dilute), heavy staggering of the hind part of the body, taking hay promptly.
6°-30'	38.3	26	60	Defæcation (faeces 8 boli, soft, mixed with pseudomembranes).
45'	—	—	—	Becoming very excited, frequently pawing, nasal sound being high, sweat secretion begins on the upper scapular region and drops along abdomen and thigh.
55'	—	—	—	Urination (urine 3.5 litres).
8°-0'	38.0	22	66	Very sensitive, pupils dilated, flank region and ribs very sensitive, hind legs frequently change position to shift body weight.
10°-45'	—	—	—	Urination (urine 2 litres).

From now on, all symptoms gradually disappear and at 5 o'clock in the afternoon he had recovered.

In general, the symptoms began 1 hour and 15 minutes after taking the bark and ended after about 22 hours, showing (1) rise of temperature, (2) dyspnoea, (3) increase of secretions and excretions and (4) paralysis of hind part of body. This experiment shows clearly the existence of some toxic ingredient in the bark, as has already been ascertained by several writers.

## Physical and Chemical Studies of the Toxin.

### I. SEPARATION OF TOXIN.

Throwing away the outer rough parts, we skinned the bark and cut it into small pieces, then dried it in the shade. This material was treated with ether, cold and hot alcohol and water.

(A) Ethereal extract, (about 5%):—The reddish brown ethereal extract has unpleasant odor and acid reaction. It scarcely dissolves in water and has not toxic properties.

(B) Alcoholic extract:—Infusum is obtained to about 15% and decoctum about 10% of the bark. They are greasy, transparent and reddish brown substances and acidic, but they do not dissolve in water and give no reaction in the animal body.

Material	Animal. No.	Weight	Mode of administration	Dosis	Result
Ethereal extract	Guinea pig. No. 13	220 gm.	Emulsion, subcutaneously	0.02 gm.	Negative
"	No. 17	250	" "	0.20	"
Alcoholic infusum	," No. 14	190	" "	0.02	"
"	Rabbit. No. 1	2585	" "	0.10	"
Alcoholic decoctum	," No. 2	2435	" "	0.10	"

Remark: Materials injected were always neutralized with dilute solution of sodium carbonate.

(C) Aqueous extract:—100 grams of the bark is shaken 3 times with cold water and then filtered. To this transparent extract acetic acid is added at the rate of 3%, then a flocy, slightly yellowish white substance precipitates abundantly. After sucking and drying, we obtained 0.18 grams of light powder. This substance (I) is soluble in water which becomes reddish brown. If we inject its neutralized aqueous solution into a Guinea-pig, the latter is affected with intoxication and the lethal dose is 0.1 gm. per 1 kilo. body weight. When the yellow, acid solution separated is treated with strong alcohol we again obtain a large quantity of flocy mass of white color. This substance (II) easily dissolves in water to a neutral reddish brown solution. It has

strong toxic action, for Guinea pigs die after a dose of 0.05 gm. per 1 kilo. body weight.

Material	Animal	Weight	Method of administration	Dosis	Result
I	Guinea pig	220 gm.	Intravenously	0.02 gm.	Exitus (strong reaction).
"	"	"	"	"	" (middle " ).
II	"	330	"	0.0165	" (strong " ).
"	"	290	"	0.02	" (" " ).

As above mentioned, the toxic constituents are not extracted with ether or alcohol, but chiefly with water, and the fraction which is soluble in dilute acetic acid but not in alcohol is more powerful than the other. The name robitin was given to this substance.

## II. PREPARATION OF ROBITIN.

The air-dried bark is shaken with 10 parts of distilled water. The extraction is twice repeated and the combined extract filtered roughly through linen cloth. The residue is pressed and its sap is also added to the filtrate. The turbid and slightly yellowish brown filtrate thus prepared is heated for 30 minutes at a temperature of 80 to 90°C. and then filtered to a transparent, reddish brown liquid. When we concentrated this filtrate to one tenth of its volume under a pressure of 20 mm. at a temperature not higher than 40°C., a saturated solution of lead acetate was added until no more precipitate occurred. After filtering the precipitate of impurities and separating the excess of lead with sulphuretted hydrogen gas, the filtrate is again condensed to one twentieth of the first volume under similar conditions. When the concentrated reddish brown extract is slowly poured into a large quantity of absolute alcohol, white flock is abundantly precipitated. After letting it stand for a night, the precipitate is sucked, washed twice with absolute alcohol and desiccated in vacuum over calcium chloride. The quantity of robitin thus prepared amounts to 3% of the air dried material.

From the liquid in which robitin was collected, we obtained a large quantity of crystals of certain potassium salts and its reddish brown mother liquor does not react on animals.

The precipitate produced by the addition of lead acetate, contains a poisonous ingredient and affects Guinea pigs. Therefore, we may assume that it perhaps contains Power's robin.

### III. PHYSICAL AND CHEMICAL PROPERTIES OF ROBITIN.

Robitin is a pure white, odorless, somewhat bitter, hygroscopic amorphous powder and easily dissolves in water and acids forming a transparent, reddish brown solution, but it is insoluble in ether, methyl alcohol, ethyl alcohol, amyl alcohol, benzene, petroleum ether, chloroform and acetone. Its aqueous solution reacts neutral and does not lose its toxicity by exposure to sunlight or heating at a temperature of 100°C. for 2 hours and half.

Robitin is glucoside but not an alkaloid or a protein.

Reagent	Acid robitin solution	
	1%	5%
Iron sesquichloride .....	negative	negative
Tannin .....	"	"
Phosphomolybdic acid .....	"	"
Sublimate .....	"	"
Potassium iodide .....	"	"
Potassium mercury iodide.....	"	"
Phosphotungstic acid.....	"	"
Tanret's reagent .....	"	"

Reaction	Robitin aqueous solution (1%)
Biuret reaction .....	negative
Xanthoprotein reaction .....	"
Million's reaction.....	"
Adam Kiewicz's reaction .....	"
Liebermann's reaction .....	"
Heating .....	"
Acetic acid .....	"
Tannin .....	"

As Power's robin is considered to be a kind of nucleoprotein, we tested the trypsin digestion for robitin, but the result was negative.

By the hydrolysis with dilute hydrochloric acid, robitin decomposes into a certain yet unknown substance and sugars which are proved by the reduction of FEHLING's solution and osazone formation.

Robitin solution		Reduction
Hydrolysed	(1%)	much
"	(5%)	"
Not hydrolysed	(1%)	negative
"	(5%)	"

The decomposition product is heated in water vapour for 1 hour with phenylhydrazin hydrochloride and sodium acetate, a large quantity of osazone is formed as bright yellowish crystals and we assumed them to be a mixture of glucosazone and rhamnosazone by their form. The quantity of sugars was estimated by PAVY-KUMAGAWA-SUTŌ's method and amounted to 19.23%.

Furthermore, glucoside robitin is decomposed by emulsin.

2% Robitin solution	1% Emulsin solution	Reduction of Fehling's solution
I 5.0 cem.	1.5 cem. (not heated)	positive
II "	" "	"
III "	1.5 cem. (heated)	negative
IV "	" "	"
V "	Water 1.5 cem.	"
VI Water 5.0 cem.	1% Emulsin sol. 1.5 cem. (not heated)	"
VII "	" " (heated)	"

Robitin contains from 2 to 3% ash, attaining sometimes to 5%. By ignition, we recognized that the ash consists chiefly of phosphor, sulphuric acid, magnesiuim, calcium, a little quantity of potassium and sodium and trace of iron and chlorine.

As in the post mortem examination we always found incomplete coagulation of blood, we made the following test to determine, whether this is caused by the haemolytic action of robitin or by suffocation.

Dilution		Volume of blood taken	1% robitin solution	Result
I	1/2	2 cem.	1 cem.	negative
II	1/4	"	"	"
III	1/8	"	"	"
IV	1/16	"	"	"
V	1/32	"	"	"
VI	1/64	"	"	"
VII	1/128	"	"	"
VIII	1/1	"	physiol. salt sol. 1 cem.	"

#### IV. THE QUANTITY OF ROBITIN IN THE BARK.

While 3 grams of robitin is obtained from 100 grams of the air-dried bark of *Robinia pseudacacia* L., its fresh bark gives 1 gram. The outer rough portion of the bark contains only one thirtieth of the inner.

### Symptomatology.

#### I. GUINEA PIG.

By intravenous injection of robitin into Guinea pigs, the following symptoms are observed: fall of body temperature (Figs. 1, 2), shivering, dyspnoea, excretion of faeces and paralysis of hind part of body. Frequently it shows lacrymal exudation, masticating movement, colic-like symptoms and spasmodic cough accompanied with vomiting and sometimes haemorrhagic diarrhoea. The Guinea pig dies after a dosis of 0.007 to 0.01 gram per 100 grams body weight (as 1 cem. solution) by intravenous injection, showing stop of respiration after 15 minutes to 3 hours.

By the hypodermic injection, almost the same symptoms are observed as by the intravenous, but they appear more slowly.

## Example I. September 27th, 1916.

Guinea pig No. 46. Body weight 180 grams.

Time	Temperature	Symptoms
1° 55'	38.4	Normal
2° 3'	—	Robitin 0.1 gram per 1 kilo. body weight, intravenously.
5'	—	Evacuation, convulsions, suddenly falls but soon get up again, dyspnœa.
7'	—	Respiration becomes quiet, above symptoms somewhat relaxing.
10'	36.0	Hind part of body becomes paralysed.
12'	—	Whole body elongates.
15'	—	Respiration and pulsation stop, exitus.

Post mortem examination: Emphysema et hæmorrhagia pulmonum, incomplete coagulation of blood.

## Example II. September 9th, 1916.

Guinea pig No. 40. Body weight 290 grams.

Time	Temperature	Symptoms
10° 25'	37.6	Normal
40'	—	Robitin 0.07 grams per 1 kilo. body weight, intravenously.
45'	—	Cough, micturition, defæcation (4 times), acceleration of respiration.
54'	—	Evacuation (5 times).
11° 2'	—	" (2 " ).
5'	36.5	" (1 " ).
12'	—	" "
20'	—	" "
24'	36.0	Slightly exhausted, slight paralysis of hind part.
25'	—	Lies down, convulsions, eye lids half opened.
36'	35.5	Strong dyspnœa.
45'	—	Head trembles frequently.
50'	—	Respiration very irregular.
54'	35.0	Spasmodic movements.
1° 40'	—	Exitus.

Post mortem examination: Emphysema et haemorrhagia pulmonum, incomplete coagulation of blood.

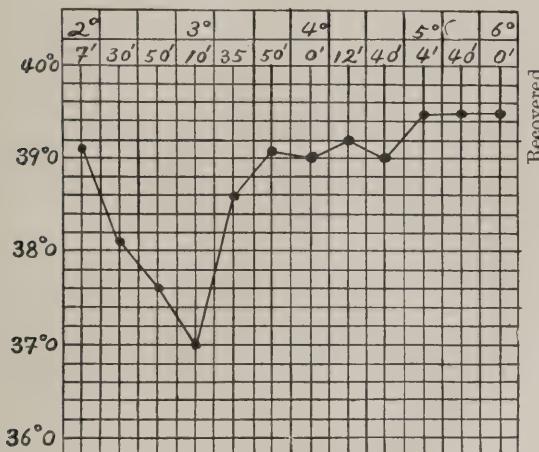


Fig. 1. Temperature curve.

Guinea pig No. 86.

Robitin 0.02 gms. intravenously at 2° 12' P.M.

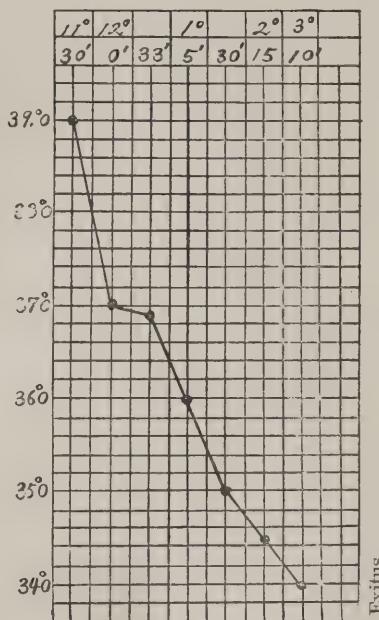


Fig. 2. Temperature curve.

Guinea pig No. 25.

Robitin 0.01 gm. intravenously  
at 11° 44' A.M.

TABLE I.

Injection of robitin to Guinea pigs.

Date	No. of animal	Body weight	Material	Method of administration	Dosis	Result
27/VI/16	4	310 gm.	Robitin A,	intravenously	0.0180 gm.	E'''
"	5	340	" "	"	0.0240	E'
14/VII	6	235	" B.	"	0.0200	E
17/VII	8	180	" "	"	0.0200	E
"	9	200	" C.	"	"	E''
18/VII	10	220	" D.	"	"	E
"	11	"	" E.	hypodermically	"	++
21/VII	12	"	" "	intravenously	"	E'''
25/VII	16	230	" "	"	"	++

Date	No. of animal	Body weight	Material	Method of administration	Dosis	Result
31/VII/16	13	200 gm.	Robitin No. I. A.	intravenously	0.0200 gm.	E'
"	14	190	" " B.	"	"	E''
1/VIII	17	250	" No. II.	"	"	E'''
"	18	220	" No. I. C.	"	"	E
"	19	230	" "	"	"	E'
2/VIII	21	250	" No. I. B.	"	"	"
"	22	"	No. II.	"	"	"
"	23	230	" No. I. B.	"	0.0100	"
"	24	"	No. II.	"	"	++
3/VIII	25	235	" No. I. B.	"	"	E''
"	26	240	" No. II.	"	"	E'
4/VIII	27	245	" No. I. B.	"	0.0050	++
"	28	240	" No. II.	"	"	"
5/VIII	29	270	" No. I. B.	"	"	E''
"	30	260	" No. II.	"	"	++
24/VIII	32	360	" No. IV.	"	0.0175	E'''
2/IX	36	310	" No. VI.	"	0.0150	E''''
4/IX	37	330	" "	"	0.0165	+
"	38	"	" "	"	"	E
"	39	380	" "	"	0.0190	++
9/IX	40	290	" No. VII.	"	0.0203	E'
"	41	340	" "	"	0.0238	++
11/IX	"	"	" "	"	0.0300	E''
20/IX	42	190	" No. VIII. A.	"	0.0133	"
"	43	"	" " B.	"	"	E'
26/IX	44	200	" No. IX. I.	"	0.0200	E
"	45	155	" " "	"	0.0105	++
27/IX	46	180	" " "	"	0.0180	E
"	47	150	" " "	"	0.0150	"
"	48	190	" " "	"	0.0190	"
"	45	155	" " "	"	0.0150	+
2/X	49	170	" No. IX. III.	"	0.0170	++
"	50	190	" "	"	0.0190	+
3/X	51	380	Robitin No. X. I. many hours in absolute alcohol	"	0.0380	++
"	52	150	Robitin No. X.	"	0.0150	E'
16/X	53	210	Robitin decolourized	"	0.0210	++
"	54	"	" "	"	"	"

Date	No. of animal	Body weight	Material	Method of administration	Dosis	Result
25/I/17	55	gm. 175	Robitin No. XIII.	intravenously	gm. 0.0200	+
"	56	190	Robitin No. XIII (heated at 100°C.)	"	"	E'''
29/I	57	200	Precipitate by Pb-acetate.	"	"	±
"	58	220	" by HCl.	"	"	+
6/II	59	190	Robitin No. XIII (heated at 100°C.)	"	"	E'''
"	60	200	Robitin No. XIII.	"	0.0300	"
"	57	"	" "	"	0.0270	E
10/II	62	"	" "	"	0.0210	±
"	63	—	" "	"	0.0300	"
19/II	64	200	" "	"	0.0300	E'''
"	65	"	" "	"	"	"
5/III	66	—	Hydrolysed solution.	"	0.0340	—
3/III	67	—	"	"	"	—
"	68	—	"	"	0.0500	±
5/III	69	—	Robitin (heated at high temp.)	"	0.0330	E'''
"	70	—	" "	"	0.0500	"
"	71	—	" "	"	0.0330	"
"	72	—	" "	"	0.0300	"
"	73	—	" "	"	0.0500	"
24/III	74	—	Precipitate by Pb-acetate.	"	0.0600	E
9/IV	76	—	Robitin.	"	0.0400	E'''

Remarks :—Negative, ± very weak, + weak, ++ middle, +++ strong reaction.  
 E Exitus in 30 minutes, E' in 3 hours, E'' in 12 hours, E''' the following day, E'''' after two days.

## II. RABBIT.

On intravenous injection of robitin, the rabbit suffers from dyspnoea, paralysis of hind part of body, depression and frequent evacuation and micturition. Lethal dose about 0.5 grams per 1 kilo. body weight.

In the post mortem examination, emphysema and haemorrhagia pulmonum and liquefaction of the intestinal content are seen, sometimes accompanied with bronchial haemorrhage and abdominal exudation.

Example. July 11th, 1916.

Rabbit No. 5. Body weight 3115. grams, female.

Time	Temperature	Symptoms
2° 30'	39.1	Normal, robitin 1 gm. (as 10 ccm. aqueous solution) intravenously.
35'	—	Head shakes, ears half hanging down.
45'	39.3	—
55'	40.1	—
3° 15'	41.0	Depressed.
20'	—	Evacuation.
30'	—	Hind part of body staggering, micturition, dyspnoea.
43'	41.8	—
4° 2'	41.5	—
30'	—	Defaecation.
40'	—	Strong difficulty in breathing, great depression, lies down
4° 50'	—	Stand up, respiratory sound very high.
5° 30'	39.2	Thenceforth no great change of symptoms, died 39 hours after the injection.

TABLE II.  
Injection of robitin to rabbits.

Date	No. of animal	Body weight kilo.	Material	Mode of administration	Dosis gm.	Result
24/VI/'16	1	2.585	Robitin	intravenously	0.72	E'''
10/VII/'17	3	2.325	"	"	0.12	††
"	4	3.115	"	hypodermically	1.00	++
12/VII	"	"	"	intravenously	"	E''''
"	3	2.325	"	"	0.50	+
22/VI/16	2	3.435	Aqueous extract of the bark.	hypodermically	50 ccm.	E''''

Remarks: For explanation of marks of results see Table I.

### III. HORSE.

If 0.0015 grams of robitin per 1 kilo. body weight is injected intravenously into the horse, we observe all the typical symptoms, and with 0.003 grams the horse falls into agonial stage, 0.05 grams being its lethal dosis.

The symptoms coincide with those of the preliminary experiment: frequent evacuation to diarrhoea, paralysis of hind parts of body, dyspnoea, hyperaemia and humidity of visible mucous membranes. The reaction caused by robitin varies in its degree not only according to the dosage, but also according to the individuality of the horse, that is, early or late appearance of symptoms, rapidity or slowness of effect and slightness or seriousness of degree. Between two horses similarly treated with robitin, the one which was given some exercise showed more serious symptoms, and the recovery was slower than in the other. If we inject intravenously 0.15 grams of robitin per 100 kilos. of horse body weight, the symptoms begin to show at 5 to 10 minutes after the injection and end at 40 to 60 minutes. After 5 to 6 hours, spirits and appetite recover, but the state of faeces continues for about 20 hours.

After the subcutaneous injection, some horses show similar reaction to that of the intravenous with same dosage, but generally the former symptoms are inferior in degree to the latter at the rate of 1:5.

Internal administration of robitin as bolus or solutio causes the same reaction to the horse as intravenous injection, but only in very large dosage.

#### Example I. November 25th, 1916.

Name of horse: Yokonami, 17 years old. Body weight 360 kilos. Nutrition good.

Time	T.	R.	P.	Symptoms.
9° 30'	37.0	9	41	Normal.
10° 0'	—	—	—	Robitin 1 gram in 5% solution intravenously.
6'	—	—	—	Hind part of body weak.
9'	—	19	—	Evacuation (faeces 30 boli, normal but small shaped.)
10° 13'	—	—	—	Defaecation (faeces about 30 boli, surface a little moist.)
20'	38.5	21	45	—
25'	—	—	—	Defaecation (faeces 40 boli, soft.)
30'	—	—	—	Visible mucous membranes moisten, legs gathering under the abdomen, hind parts of body stagger strongly.
36'	—	—	—	Cough.
38'	38.5	21	48	"
40'	—	—	—	" (many times).
42'	—	—	—	" (2 times, short and humid).

Time	T.	R.	P.	Symptom
10° 44'	—	—	—	Cough, lacrymation, nasal discharge, colic-like symptoms.
46'	—	—	—	Cough (2 times), spasmodic contraction of skin muscles, dyspnoea.
49'	—	—	—	Cough (2 times).
52'	—	—	—	Diarrhoea
11° 1'	—	—	—	Defaecation (muddy faeces, 2 litres), farts frequently.
5'	—	—	—	Dyspnoea, lies down.
10'	—	—	—	Frequent nausea.
12'	38.5	24	49	Conjunctiva shows strong hyperaemia.
15'	—	—	—	Spasm of the whole body, nasal sound is very high, pulsation rises, falls, almost in agony.
30'	—	—	—	Again get up
2° 0'	—	—	—	Depressed.
3° 0'	38.0	18	43	—
4° 30'	—	—	—	Recovered.

## Example II. June 26th, 1917.

Name of horse: Kohiko, 18 years old. Body weight 500 kilos, a little fat.

Time	T.	R.	P.	Symptoms
1° 10'	37.8	20	48	Slight hyperaemia of conjunctiva, nothing special.
25'	—	—	—	0.7 grams robitin in 3% aqueous solution intravenously.
10° 26'	—	—	—	Excited, salvation, accelerated respiration.
27'	—	—	—	Evacuation (faeces 10 boli, slightly soft), colic-like symptoms to be seen.
28'	37.9	30	48	Evacuation (faeces 15 boli, soft), licks the floor.
32'	—	—	—	Diarrhoea (2 litres, somewhat lumpy), very uneasy, rubbing its body on the side wall.
35'	—	29	48	Diarrhoea (1 litre), peristalsis very excited.
1° 38'	—	—	—	Watery diarrhoea (about 3 litres).
43'	—	35	—	" . . . " ( " . . . " ).
50'	—	—	—	Exhausted, depressed.
2° 0'	—	—	—	Almost recovered.

## Example III. February 15th, 1917.

Name of horse: Meikō, 21 years old. Body weight 390 kilos, nutrition good.

Time	T.	R.	P.	Symptoms
11° 20'	38.0	10	36	Normal.
29'	—	—	—	0.5 grams robitin in 2% solution intravenously.
11° 33'	—	—	—	Hind parts of body weak, dyspnoea with high nasal sound.
37'	—	—	—	Evacuation (muddy faeces 1 litre), penis often lowers.
40'	38.2	20	40	—
47'	—	—	—	Defaecation (faeces 15 boli, very soft.)
54'	—	—	—	"      (muddy faeces 2.5 litres.)
1° 24'	—	—	—	Diarrhoea (2 litres).
37'	—	—	—	Micturition (about 3 litres).
2° 0'	—	—	—	Recovered.

## Example IV. December 10th, 1916.

Name of horse: Senjin, 21 years old. Body weight 340 kilos. Nutrition middle.

Time	T.	R.	P.	Symptoms
10° 50'	37.5	15	40	Normal.
11° 5'	—	—	—	0.5 grams robitin in 1.5% solution subcutaneously.
14'	—	—	—	Farting, peristalsis excited, rumblings to be heard.
24'	—	—	—	Defaecation (faeces 30 boli), very uneasy.
11° 30'	—	—	—	Frequent pawing.
44'	—	45	—	Defaecation (faeces 40 boli, surface very moist), breathing accelerates.
48'	—	—	—	Evacuation (faeces 15 boli, very soft, mixed with mucus).
50'	37.8	40	43	A little depressed.
55'	—	—	—	Defaecation (muddy faeces 1.5 litres) uneasy.
12° 0'	—	—	—	Gradually recovered.

TABLE III.  
Intravenous injection of robitin to horses.

Date	Name of horse	Age	Material	Dosis	Reaction
13/XI/16	Shunketsu	16	Robitin No. XII.	gm. 0.5	strong
15/XI	Hisatori	22	" "	"	"
17/XI	"	"	" "	"	negative
"	Yokonami	17	" "	"	strong
18/XI	"	"	" "	"	negative
28/XI	Shunho	"	" No. VII.	"	strong
1/XII	Kasuga	21	" "	0.4	"
6/XII	Seiten	19	" No. XII.	0.5	"
8/XII	Senjin	21	Robitin, decolor. with animal charcoal.	0.2	negative
19/I/17	Ebara	16	Robitin No. XIII. decolorized.	0.5	strong
21/I	Kôgetsu	17	Robitin No. XIII.	1.5	very strong
27/I/17	Renshô	20	" "	0.6	strong
3/II	Setagaya	19	" No. XIV.	0.4	weak
25/II	Netani	14	" No. XV.	0.5	strong
17/II	Komori	—	" "	0.2	negative
23/II	Meikô	21	" No. XVI.	0.5	weak
"	Seiten	19	Robitin (boiled the sol. at 100°C.)	"	middle
"	Kenshu	21	" "	"	strong
24/II	Seiten	19	" "	"	negative
16/III	Kokkô	8	Robitin	"	strong
17/III	Senjin	21	"	"	"
19/III	Kokkô	8	"	"	"
28/IV	Netani	14	"	"	weak

TABLE IV.  
Subcutaneous injection of robitin to horses.

Date	Name of horse	Age	Material	Dosis	Reaction
8/XII/16	Kasuga	21	Robitin No. XII.	gm. 0.5	very strong
13/I/17	Meguro	—	" No. XIII.	"	middle
20/I	Hatsugiku	12	Robitin No. XIII. decolorized.	"	strong
16/III	Senjin	21	Robitin No. XIV.	1.0	"

Date	Name of horse	Age	Material	Dosis	Reaction
10/V	Furumatsu	15	Robinin No. XV.	gm. 2.0	weak
8/VI	Shômei	13	" "	1.0	"

TABLE V.  
Internal administration of robinin to horses.

Date	Name of horse	Age	Material	Dosis	Form of preparation	Reaction	Remarks
19/III/17	Yamacha	15	Robinin No. XVI.	gm. 3.0	Bolus.	middle	Reactions slowly appeared.
21/III	Senjin	21	" "	5.0	"	weak	"
4/V	Furumatsu	15	" "	"	Solutio (200 cem.)	"	"
7/V	Komaba	—	" "	10.0	" (400 cem.)	strong	"

#### IV. CATTLE.

The reaction observed by the intravenous injection of robinin to cattles is almost the same as to horses, namely, hyperæmy of the visible mucous membranes, fatigue, discouragement, increase of secretion and excretion, dyspœa and paralysis of hind part of body.

Example. June 2nd, 1917.

Cow. 3 years old Ayrshire, nutrition wrong.

She was injected a kind of abortus bacillus about 6 month ago.

Time	T.	R.	P.	Spmptoms
1° 0'	38.4	40	50	She made violently noise when restrained, without specialty.
1° 15'	—	—	—	4 grams robinin in aqueous solution intravenously.
20'	—	68	—	Breathing accelerate, a little discouraged.
35'	—	80	—	—
38'	—	90	54	Micturition (1 litre).
45'	38.8	—	—	Defæcation (1 litre. muddy).
46'	—	—	—	" (0.5 litre, muddy), conjunctival hyperæmy.

Time	T.	R.	P.	Symptoms
55'	38.8	106	56	Defæcation (0.5 litre, muddy), tenesmus.
2° 0'	—	—	—	" (1 litre, muddy).
4'	—	110	—	Body leaning on the side wall.
10'	—	—	—	Diarrhoea (1 litre).
15'	38.4	94	64	Watery diarrhoea.
25'	—	—	—	Diarrhoea.
26'	—	80	—	Watery diarrhoea, breathing becomes quiet, thenceforth symptoms gradually disappeared, but to to-morrow morning sometimes diarrhoea.

### Summary.

1. A new toxic glucoside, named 'Robitin', was isolated from the bark of *Robinia pseudacacia* L. Its quantity in the fresh bark amounts to 1%.
2. The intoxication by robitin is recognized in cattle, horse, rabbit and Guinea-pig. The chief symptoms are dyspnœa, increase of secretions and excretions and paralysis of the hind part of the body. In the post mortem examination, we observe emphysema and haemorrhagia of lungs and incomplete coagulation of the blood.
3. Robitin causes toxic reaction by doses of 0.0015 grams in horses, 0.02 grams in cattle, 0.07 grams in Guinea-pigs and 0.5 grams in rabbits per 1 kilo. body weight.
4. The reactions observed by the injection of robitin into horses agree exactly with the symptoms in the experiment with the fresh bark.

We have to express to Assistant Prof. T. SHIMAMURA our great indebtedness for his advice so kindly given during the progress of the work.

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All communications relating to this Journal should be addressed to the Director of the College of Agriculture.

# The Spermatogenesis of Domestic Mammals.

## I. The Spermatogenesis of the Horse (*Equus caballus*).

BY

Kiyoshi Masui.

(From the Laboratory for Agricultural Zoology.

Director : Prof. C. ISHIKAWA.)

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With Plates XI-XIII and two Text-Figures.

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In the present paper I intend to describe the entire spermatogenesis of the horse only, but I hope to continue my studies on the germ cells of other domestic mammals. The points of special interest in the spermatogenesis of the horse are:—the presence of the accessory chromosome, the mode of formation of the synapsis, the reduction of the chromosomes, the development of the spermatozoa, the fate of the mitochondria and the behavior of the chromatoid corpuscles.

The work was done under the direction of Professor CHIYOMATSU ISHIKAWA, to whom I here desire to express my sincere gratitude. Thanks are also due to Mr. SUZUKI, who kindly supplied me with materials.

### I. Materials and Methods.

The testes of horses at different ages were used. The material was chiefly obtained from the Shirakawa Branch of the Remount Dépôt and from the Veterinary College of the Department of War.

For preservation, it is necessary, as is already known, that the testes should be cut into small pieces and then put into the fixing reagents. Four fixatives were used; FLEMMING's strong fluid, CHAMPY's fluid, BOUIN's fluid

and ZENKER's mixture. Of these, both FLEMMING'S and BOUIN'S fluid gave satisfactory results, while the mitochondria were beautifully brought out by CHAMPY'S fluid. The sections were cut  $5-10\ \mu$  in thickness. For staining HEIDENHAIN'S iron-haematoxylin, DELAFIELD'S haematoxylin and FLEMMING'S triple stain (safranin, gentiana violet and orange G) were chiefly used. For the staining of the mitochondria a modification of BENDA'S method was employed, but no precise differentiation was obtained, while HEIDENHAIN'S iron-haematoxylin stained them distinctly, when the preparations were previously treated with CHAMPY'S fluid. To differentiate the chromosome nucleolus OBST'S method was employed, but did not give satisfactory results.

The development of the tubules of the testis is quite different at different ages of the animals. In very young animals, spermatogonial cells in various stages and many Sertoli-cells are found along the wall of the tubule (Fig. 1). In animals a little older (Figs. 2, 3) the tubules usually contain spermatogonia, young spermatocytes and Sertoli-cells. In mature animals, a few spermatogonia and Sertoli-cells are found along the wall of the tubules; next to these the spermatocytes in various stages, the spermatids and the unripe spermatozoa appear (Figs. 5, 6). Toward the center of the tubule some ripe spermatozoa are found.

## II. The Spermatogonia.

In the testes of adult individuals we can distinguish two generations of spermatogonia, a primary and a secondary (Figs. 7, 8, 11, 12). The primary spermatogonia (penultimate spermatogonia) are more abundant in the immature testes of young individuals (Fig. 1). Their nuclear organization and their cytoplasmic structure are similar to those of the secondary spermatogonia (ultimate spermatogonia), but larger and less in number in comparison with the secondary spermatogonia (Figs. 7, 12). The resting nucleus of both spermatogonial generations usually contains one large nucleolus and several small chromatin masses (Karyosomes) (Figs. 7, 8).

The earlier generation of the nutritive cells (Sertoli-cells), as shown in Fig. 1, is similar to the resting spermatogonia, but the latter contain many chromatin granules.

MONTGOMERY ('11 b) succeeded in tracing directly the formation of Sertoli-cells from the antepenultimate spermatogonia in man. The close resemblance of Sertoli-cells and spermatogonia, and their relative number, led BACHHUBER ('16) to the conclusion that the nutritive cells in the rabbit arise from the primordial germ cells.

During the early prophase of the spermatogonia numerous chromatin masses appear which gradually increase in size. The nuclei in this stage greatly resemble those of the interstitial cells, both in appearance and in size (Fig. 3). Fig. 9 represents a prophase of a primary spermatogonium. The chromosomes here are variously curved and somewhat elongated but one of them usually appears round or oval in form.

In the equatorial plates of the metaphase of the spermatogonia the chromosomes have a tendency to collect together and form a mass, so closely overlying one another that it is not possible to identify the individual chromosome (Figs. 4, 10). From the preparations of these stages, it is impossible to obtain a definite conclusion with regard to the number and the condition of the chromosomes. In favorable preparations I was able to count over thirtythree chromosomes, but never more than thirtyeight. Thus, although it is difficult to determine the accurate number of chromosomes, it is easy to distinguish many symmetrical pairs as shown in Fig. 4. In the metaphase all the chromosomes arrange themselves at the equatorial plate, where they simultaneously divide into two portions; no special chromosome with different behavior is to be seen among them (Figs. 4, 11, 12). During the prophase, as mentioned above, one of the chromosomes usually appears round or oval in form and this may represent the future accessory chromosome (Fig. 9). It is, however, impossible to establish direct continuity between the chromatin nucleolus of the spermatogonium and the future accessory chromosome.

As to the essential achromatic structure of the spermatogonia, satisfactory preparations are very difficult to obtain. In the earlier stage of the spermatogonia, the cell boundaries are indistinct and the amount of the cytoplasm, especially in the secondary spermatogonia, is very small. The idiozome rarely appears, and the centrosome as well as the other cytoplasmic structures can not be identified (Figs. 7, 8).

### III. The Primary Spermatocyte.

#### A. GROWTH PERIOD.

*Resting stage* :—The daughter cells resulting from the last division of the spermatogonia are the primary spermatocytes and these immediately go over to form the resting stage. The primary spermatocytes in the resting stage rarely appear in the testes of young animals (Fig. 2). These are always in contact with the spermatogonia arranged directly within the wall of the tubule and considerably smaller than the latter cells (Fig. 3). The nucleus of the spermatocyte in the resting stage usually contains, like that of the spermatogonial cells, one large chromatin nucleolus and several small chromatin masses (Fig. 13). The chromatin nucleolus has an indistinct contour and stains intensely with iron-haematoxylin, the same colours staining the small chromatin masses similarly (Figs. 13, 14). The cytoplasmic structures, with exception of the idiozome, can not be identified (Fig. 13). In the succeeding stage many small chromatin masses begin to appear (Fig. 15).

*Synaptic stage* :—After a brief resting stage of the primary spermatocyte the chromatin soon condenses into an apparently continuous, slender filament, and passes to the leptotene stage (Fig. 16). The leptotene threads later begin to converge towards one side of the nucleus, while the bulk of the cell gradually increases to about twice the size of the resting primary spermatocyte (Fig. 17). Finally the chromatin filaments converge at a pole of the nucleus where the idiozome is situated (Fig. 18), and at the same time the nuclear wall expands leaving a clear space on the other side of the nucleus. In such cells, as shown in Fig. 18, we can clearly see the parallel arrangement of the chromatin threads. The centrosome can not be traced within the idiozome at this stage.

Near the end of this stage the whole mass of the chromatin threads gradually moves toward the center of the nucleus, the large clear space thus gradually disappearing, while the nuclear wall becomes spherical and more clearly defined (Fig. 19).

During the synaptic stage the chromosome nucleolus is invariably located at the converging point of the chromatin threads and retains its sharp contour but is never found in the clear area (Figs. 17, 19).

*Post-synaptic stage* :—The conjugated chromosomes as stated above gradually move towards the center of the nucleus, while the bulk of the cell increases. These proceed more and more until the chromatin spireme spread slowly throughout the nucleus, becoming more loosely situated, the individual filament growing thicker but staining fainter.

During the Post-synapsis the accessory chromosome retains its staining capacity and its sharp contour (Figs. 20—22). Fig. 20 is drawn from a strongly destained preparation where the accessory chromosome is stained intensely with iron-haematoxylin while the chromatin spireme is very faintly stained. In the post-synapsis the accessory chromosome usually remains in contact with the nuclear membrane and generally appears oval, it varies in size and form, sometimes being heart-shaped and occasionally distinctly bipartited (Figs. 22—28).

In the growth stage one, two or sometimes three small deeply stained granules make their appearance in the cytoplasm (Figs. 22, 23, 25), these are similar in form and behavior to those described by WILSON ('13) in *Pentatomata*.

In the primary spermatocyte the idiozome is a conspicuous body, placed close to the nuclear wall (Figs. 19, 20, 21, 22, 25, 26). During the synapsis it takes its position at the pole of the nucleus, where the chromatin spireme converges (Figs. 18, 19). It has not, however, a distinct boundary, and is represented only as an oval or a round mass. It does not change its position in the early post-synapsis but becomes homogeneous in its appearance (Figs. 19, 20). In the prophase of the first reduction division, it is divided into two small spheric bodies which gradually move apart from each other. During the synapsis and post-synapsis the centrosome, with rare exceptions, can not be found within the idiozome (Figs. 22, 25, 26).

*Prophase* :—Finally the spiremes become shortened in length, and form many variously curved chromosomes, but the rings or loops as described in other forms at this stage do not appear (Figs. 28—30). Meanwhile the idiozomes begin to disintegrate, becoming more and more granular, while the centrosome appears distinctly close to the nuclear wall (Fig. 29). This latter soon disintegrates and the chromosomes arrange themselves in the metaphase plate (Figs. 31).

As stated before, it is not possible to make out the individual chromosomes during the prophase, since they overlie one another, but among the curved chromosomes an oval or heart-shaped accessory chromosome can be found which usually takes its position at a point outside the ordinary chromosomes (Figs. 29, 30). This odd chromosome can be identified by its shape and behavior in all the succeeding stages of first reduction division. In the final prophase the chromatoid corpuscles are always situated among the chromosomes (Fig. 30) but can be easily distinguished from the latter by their small size. Finally the mitochondria become scattered throughout the cell body in this stage.

#### B. THE FIRST REDUCTION DIVISION.

*The number of chromosomes:*—As in the case of the spermatogonia, the chromosomes also have a tendency to mass together in the metaphase plate of the first reduction division. On account of their haploid number and largeness in size, it is not, however, difficult to distinguish them individually and so to count their number. For this investigation preparations which are stained with iron-haematoxylin and FLEMMING's triple dyes are used. With iron-haematoxylin it is necessary to decolorize the preparation strongly, as otherwise the boundaries of the chromosomes can not be distinguished. Careful examination of good polar views of the spindles of more than fifty preparations of the first reduction division convinced me of the presence of eighteen chromosomes in the equatorial plate in one half of them and nineteen in the other half (Figs. 32—36). This difference in number is to be accounted for by the presence or the absence of the accessory chromosome in the one or the other. In those cells in which nineteen chromosomes are found we have eighteen bivalent and one univalent accessory chromosome (Figs. 35, 36).

Besides, the chromosomes show distinct and constant differences in size. In those cells in which eighteen chromosomes are counted, we can distinguish four large-, ten middle- and four small-sized ones (Text-fig. 1 *b*). In the other half number of the cells which contain nineteen chromosomes we have four large-, eleven middle- and four small-sized ones (Text-fig. 1 *a*). Thus it is one of the eleven middle-sized chromosomes, that is to be accounted for as an accessory.



Text-fig. 1. Chromosomes of metaphase plate of first reduction division.  
*a*, nineteen chromosomes (four large-, eleven middle- and four small-sized ones);  
*b*, eighteen chromosomes (four large-, ten middle- and four small-sized ones).



Text-fig. 2. Side view of chromosomes in the first reduction division.

*The reduction* :—The eighteen bivalent chromosomes become arranged in the equatorial plate of the first maturation spindle with their short axis parallel to that of the spindle (Figs. 37, 40, Text-fig 2.) The point of conjugation of two univalent chromatin spiremes thus lies in the equator of the spindle, where the division takes place (Figs. 37, 40, 41, 43). No longitudinal split of the chromosomes, therefore, can be recognised, and no typical tetrads are formed in this stage (Figs. 31, 37, 43).

longitudinal split of the chromosomes, therefore, can be recognised, and no typical tetrads are formed in this stage (Figs. 31, 37, 43).

*The accessory chromosome* :—In the metaphase of this division (the first reduction division) the oval or the heart-shaped odd chromosome moves undivided toward one pole of the spindle in advance of the ordinary chromosomes (Figs. 38, 39, 43). Sometimes it can be distinguished from the other chromosomes even at the anaphase (Fig. 44), but it usually becomes indistinguishable later, when the chromosomes collect so closely together that their individual outlines are entirely lost to view. It is, however, certain that one half of the daughter cells thus formed contains the accessory chromosome, while the other half does not.

#### IV. The Secondary Spermatocyte.

*The resting stage* :—As the resting stage of the secondary spermatocyte is rarely found in the horse, the duration of this stage appears to be very brief. A similar condition is recorded by WODSEDALEK ('13) in the pig, by JORDAN ('11) in the opossum, and by STEVENS ('11) in the guinea-pig, but curiously enough WODSEDALEK ('14) denies the occurrence of this stage in the horse.

Approximately one half of the cells of the secondary spermatocytes in the resting stage contain one large and several small chromatin masses (Figs. 47,

49), while the cells of the other half have only the small chromatin masses (Figs. 48, 50). We thus have a phenomenon of dimorphism in this stage in regard to the existence of the chromatin nucleolus. In the cells where this latter structure occurs, it is usually found closely located to the nuclear wall (Fig. 49).

Both the idiozome and the mitochondria are distinctly seen in this stage (Figs. 49, 50). But the centrosome is rarely seen within the idiozome (Fig. 49).

*The second reduction division* :—In this division all the chromosomes arrange themselves to form the equatorial plate, where they show a tendency to gather into a mass, as in the case of the first reduction division. But in fairly good stained preparations I have been able to find the accessory chromosome and also make out the ordinary ones individually (Figs. 52—54). In consequence of the first reduction division one half of the equatorial plates of this division contains eighteen chromosomes, while the other half has besides these one accessory chromosome (Fig. 52).

The accessory chromosome is sometimes placed apart from the ordinary chromosomes in the equatorial plate, and is usually larger than these (Fig. 52).

The second pairing of the chromosomes in the second reduction division is reported to occur: by JORDAN ('11) in the opossum, by GUYER ('10) in man, and by WODSEDALEK ('13) in the pig. In the horse WODSEDALEK ('14) found that the second pairing of chromosomes takes place immediately after the first division. His reason for the occurrence of this phenomenon is, that "the resulting nine chromosomes are not one half the size of the original chromosomes of these cells, but exactly of the same size and apparently quadrivalent." I have failed to find the second pairing of the chromosomes in my preparations. But sometimes incomplete fusion of the chromosomes is seen to occur, as JORDAN reported in the opossum, and in such cases occasionally fourteen or more chromosomes can be counted (Figs. 51, 54). Whether this represents the secondary pairing, I am not able to determine. Fig. 57 represents an anaphase of the second reduction division, showing no lagging or advancing chromosomes such as GUYER ('10) has described for man and WODSEDALEK ('14) for the horse. In this division the accessory chromosome is divided at the same time with the ordinary chromosomes (Figs. 55—58).

From the appearance of the chromosomes it is evident that the real character of the second division in the case of the horse is simply an ordinary mitosis, the accessory chromosome being also divided into two, like the ordinary ones.

## V. The Spermatid.

From what is stated above, it is obvious that one half of the spermatids contains eighteen chromosomes, the other half nineteen (eighteen ordinary chromosomes plus one accessory). Immediately after the second division, the chromosomes begin to break up and the nucleus enters into the resting stage (Figs. 58, 59).

The spermatids in the resting stage contain several chromatin masses (Figs. 60, 61, 65) and thus a phenomenon of dimorphism in this stage in regard to the existence of the chromatin nucleolus can not be determined. GUYER ('10) has, however, found the chromatin nucleoli in one half of the spermatids in man and believes that these nucleoli stand in direct genetic continuity with the two eccentric chromosomes seen in the spermatogonia, the two chromatin nucleoli and the accessory chromosome of the spermatocytes. Similarly WODSEDALEK ('13) in the pig and BACHHUBER ('16) in the rabbit report the presence of an accessory chromosome in one half the number of the spermatids. On the other hand STEVENS ('11) found that in the guinea-pig the spermatids and the spermatozoa are not visibly dimorphic. A similar condition was found by JORDAN ('11) in the opossum, where he says that "the accessory disappears with ordinary chromosomes into the nuclear reticulum of the resting spermatid. In later stages the nucleus contains a conspicuous central plasmosome, but all trace of the accessory chromosome seems henceforth lost."

During the resting stage of the spermatid the centrosome distinctly appears near the idiozome (Figs. 61, 63). Fig. 59 shows the telophase of the second reduction division in which the centrosome appears at one pole of the cell in close contact with the nuclear wall, while the mitosome (*Spindelrestkörper*) and the mitochondria take their position at the opposite side of the nucleus in the cytoplasm.

## VI. The Transformation of Spermatids.

The spermatids, after passing through a brief resting stage, begin to develop into spermatozoa and the chromatic materials soon collect on the surface of the nucleus (Figs. 63, 64).

The important changes which occur during the transformation of the spermatids are those connected with the centrosome. With the formation of the mass of the mitochondria, the centrosome, which up to this time remained as a single body, begins to divide (Figs. 63, 65). Of these two centrosomes thus formed, the one which is somewhat rod-shaped, comes to be placed in contact with the nuclear wall, while the other, which has a spherical shape, remains for a time near the periphery of the cell, the two being connected by a fine filament (Fig. 63). Soon after this the peripheral centrosome again divides (Fig. 65), and three bodies are thus formed, the original central one lying close to the nuclear wall, and the inner and outer are formed of the parts of the peripheral centrosome. Of these latter two, the inner one travels toward the central centrosome, and comes to be placed in close contact with it, while the outer remains at the place of its formation and changes its shape to a disk or a sphere (Fig. 67). Meanwhile a fine filament is seen to proceed from the inner of the peripheral centrosomes backward towards the surface of the cell, which later becomes the axial filament of the spermatozoon (Fig. 68). Together with the division of the centrosome the archoplasm appears first near the mitochondrial mass, but soon moves alongside the nuclear wall, to occupy its final position directly opposite to the mitochondrial mass (Figs. 66, 67).

In the horse I could not follow directly the origin of the archosome either from the idiozome or independently in the cytoplasm. WODSEDALEK ('13) states that in the pig the archosome is formed from the sphere, just as MEVES (1899) showed long ago in the guinea-pig, the sphere of the spermatid according to MEVES is homologous with the idiozome of the spermatogonium or spermatocyte.

At any rate the sphere thus formed finally passes to occupy its position at the apex of the unripe spermatozoon and there becomes the

perforatrium just as WODSEDALEK has shown to be the case in the pig (Figs. 71-73).

With the migration of the sphere the cell becomes elongated and the nucleus shifts its position near to the anterior end of the cell, while the cytoplasm collects at its posterior portion (Figs. 66-71). Most of the chromatic material now collects more and more near the periphery of the nucleus (Figs. 65-67), and the anterior centrosome comes so close to the nuclear wall as to be placed in a small depression which meanwhile appears at the posterior end of the same (Figs. 70, 71, 73). The bulk of the inner centrosome of the peripheral ones passes into the formation of the axial filament, and the centrosome as such finally disappears. The disc shaped or round posterior centrosome gradually increases in size and the axial filament extends backward, directly passing through it (Fig. 72). After the projection of the tail out of the cell, this posterior centrosome diminishes in size and moves apart from the anterior (Figs. 70, 71), until it becomes entirely lost to view (Figs. 73, 75). Whether this centrosome is thrown off together with a big mass of cytoplasm, during the final development of the spermatozoa as is described by WODSEDALEK ('13) in the pig, is in my preparations not determined.

The general behavior of the centrosomes in the development of the spermatozoa in the horse is similar to that in man and in the rabbit, as stated by MEVES (1898). Finally the chromatoid corpuscles are cast off together with a mass of cytoplasm, and the mitochondria develop to form the middle piece (Fig. 73).

The ripe spermatozoa found free in the lumen of the tubule, appear to vary considerably in size. This, however, is owing to the fact that the head of a ripe spermatozoon is flattened, and so the lateral view gives quite a different impression to that of the flattened side (Figs. 74, 75).

## VII. Chromatoid Corpuscles.

It is not possible to positively identify the chromatoid corpuscles until after the postsynapsis. But as stated above, in a stage immediately following the synapsis, small granules make their appearance in the cytoplasm closely

in contact with the nuclear wall (Figs. 22, 23). Sometimes two or three very small granules are found near a large one, the former, however, usually disappearing in later stages. Bodies similar to these were found by many investigators in some mammals and in lower animals, and received the name of chromatoid corpuscles. But their origin and function are still a question. Although they stain intensely either with iron-haematoxylin or DELAFIELD's haematoxylin, it is not possible to determine whether they originate within the nucleus or in the cytoplasm.

During the prophase of the first reduction division the chromatoid corpuscle, i. e. the one that remains, rapidly enlarges and becomes more prominent, and comes to be placed close to the chromosomes as the nuclear membrane disappears, but is always distinguishable from them by its smaller size (Fig. 30).

During the first reduction division sometimes one or two extra small bodies again make their appearance (Figs. 42, 44, 45), which in the anaphase do not divide, but usually lie close to the spindle, often directly upon it and sometimes within it (Figs. 39, 44, 45). These, except in very rare cases, disappear in the telophase, and when the new cell wall is formed in the daughter cell it remains with the mitosome (PLATNER) or Spindelrestkörper (MEVES) close to the cell-plate (Fig. 46). The secondary spermatocytes are, as said before, of two kinds, the one with and the other without the chromatoid corpuscle. During the resting stage of the same it attains its maximum growth, and sometimes a vacuole appears in it (Figs. 49, 51). Its position in this stage is quite variable, but it is mostly placed near the cell wall (Figs. 49—51).

In the second reduction division the chromatoid corpuscle repeats the same behavior as in the first, but the extra bodies were not found in this division (Figs. 57, 58), and as it also passes without division into one of the daughter cells (Fig. 58), it is found approximately in one-fourth of the total spermatids. Its position in early stages of the spermatids is not fixed, but it is situated more commonly on the posterior side of the nucleus (Figs. 61, 63, 64), and when the elongation of the tail begins it moves further backward from the nucleus to find its final position in the middle region of the tail (Figs. 68, 71). Its fate in succeeding stages is not determined but it is most

probably cast off with the main portion of the cytoplasm out of the body of the spermatozoon.

### VIII. Mitochondria.

The mitochondria appear in small numbers throughout the cytoplasm in the growth stage (Figs. 23, 24) and are recognisable in all the succeeding stages up to the development of the spermatozoa.

In the prophase of the first reduction division they are still found scattered throughout the cytoplasm. In the metaphase of the first reduction division the mitochondria remain undivided and lie outside the spindle (Figs. 38, 39), but after the division they seem to be approximately equally distributed in the daughter cells. In the resting stage of the secondary spermatocyte the mitochondrial granulations are also seen scattered all over the cell body, but a portion of them group together and lie near the idiozone which now appears in the cell (Figs. 49, 50). Their behavior in the second reduction division is similar to that in the first division.

In the spermatid most of the mitochondria group together and lie at the posterior part of the cell body while the rest of them still lie scattered in the cell body (Fig. 62). The fate and behavior of the mitochondria during the development of the spermatozoa are very significant. Simultaneously with the division of the centrosome the mitochondria which lie at the posterior part of the cell body commence to fuse together and form a mass which is similar to the "Nebenkern" in insects (Figs. 62, 69). But this mitochondrial mass is very indistinct, having no sharp contour as compared with those seen in the lower animals (Fig. 69). Its location at this stage is the posterior part of the cell (Figs. 62, 69), where it finally encloses the axial filament and the centrosomes (Fig. 69), and thus comes to occupy the middle piece of the spermatozoa (Fig. 73). During these changes the granulations gradually disappear and it becomes more homogeneous (Figs. 74, 75). A small portion of the mitochondria which lie scattered in the cell body in the early stage of the spermatid is probably cast out of the body of the spermatozoon (Fig. 73).

## IX. General Consideration.

### A. THE ACCESSORY CHROMOSOME.

An odd or unpaired chromosome also exists in the male germ cells of the horse as in many other animals. Its form and behavior correspond almost exactly with the body termed by WILSON the heterotropic or accessory chromosome, so that I do not hesitate to identify it with the same. The accessory chromosome, however, can not be recognized during the division of the spermatogonia; it probably divides into two like the ordinary chromosomes, but comes in view during all succeeding stages. During the prophase of the first maturation division, it is situated outside the chromosomes, as an oval body, but sometimes it presents a rod or heart-shape, or rarely bipartited form, and during the division it passes undivided to one pole of the spindle in advance of the ordinary chromosomes. In the cells of the secondary spermatocytes in which it is present it assumes the form of a chromosome nucleolus, and divides into two like the ordinary chromosomes, to enter into the two daughter cells, but it can not be traced in the spermatid. The occurrence of an accessory chromosome in vertebrates, as I am aware, was first recorded by GUYER in the guinea-fowl ('09), in the domestic fowl ('09), and in man ('10). This was soon followed by JORDAN ('11) who found the same in the opossum, STEVENS ('11) in the guinea-pig, WODSEDALEK in the pig ('13) and in the horse ('14), BACHHUBER in the rabbit ('16) and YOCOM ('17) in the mouse. All these investigators, with the exception of GUYER in the common fowl ('16), believe that both classes of spermatids, the one with an accessory chromosome and the other without it, develop into spermatozoa.

WODSEDALEK ('13) found two accessory chromosomes in the male germ cells of the pig and was convinced of the existence of dimorphism among the spermatozoa, the one with and the other without the accessory, and by careful examination of the male and female somatic cells of the embryo he also found the existence of a dimorphism in the number of chromosomes in these cells, from which he concludes that "the result of the present investigation (his investigation), therefore, adds support to the chromosome theory of sex-determination, since they shew that in the vertebrates, as well as in some of

the lower forms, there exists a dimorphism in number of chromosomes in the somatic as well as the germinal cells of the two sexes. It is highly probable that a condition similar to those found in the pig, as regards sex-determination, exists in man and in the other vertebrates which possess the accessory or x-chromosome."

During the resting stage of the spermatogonia usually one, sometimes two large nucleoli are invariably present. According to GUYER ('10) the chromatin nucleolus of the spermatogonia and the accessory chromosome are one and the same. WODSEDALEK is of the same opinion as regards the pig ('13) and the horse ('14). BACHHUBER ('16) also found two or more large, spherical Karyosomes in the rabbit and believes that these may be two accessory elements which can be traced very accurately after the formation of the primary spermatocytes.

From all appearances of the spermatogonia in the horse one is led to believe that the chromatin nucleolus in the spermatogonium is not the future accessory chromosome.

#### B. SYNAPSIS AND THE REDUCTION DIVISION.

As already described, in post-synapsis the chromatin spiremes appear in about half the original number. From this fact it is conceivable that the conjugation of the chromosomes probably occurs during the synaptic stage. But it is difficult to determine definitely whether the conjugation of the chromosomes takes place by parasyntapsis (side by side conjugation) or telosyntapsis (end to end conjugation). In vertebrates most of the investigators have reached the conclusion that the conjugation of the chromosomes occurs by parasyntapsis [WINIWARTER ('02), SCHREINER ('06) and WILSON ('12)], while JORDAN ('11) believes that in opossum the first numerical reduction of the diploid group of chromosomes occurs by telosyntapsis (metasyndesis).

In the horse there is no evidence of the occurrence of telosyntapsis. During the synaptic stage the longitudinal duality of the chromatin spiremes can clearly be seen and this duality is not the longitudinal split of the chromosome as seen in the chromosomes of the somatic cells in other animals. From accurate observations, however, it is evident that the longitudinal duality is simply due to the parallel arrangement of two univalent spiremes. Nearly at the end of the synaptic stage the parallel arrangement of chromatin

spiremes can not be seen, but they appear in about half the original number and twice as thick as those of the leptotene stage. Judging from these facts it seems most probable that conjugation of the chromosomes probably takes place by parasympapsis. Moreover, it is striking evidence in support of the conception of parasympapsis that during the synaptic stage partial fusion of two spiremes, which are arranged in pairs, frequently occurs (Fig. 18).

The bivalent chromosomes thus united during the synaptic stage are so placed in the equatorial plate of the first division that their long axis is at right angle to that of the spindle (Text-fig. 2). The division begins to occur at the end of the chromosomes where the spindle fibres attach, and soon become separated into their components (Text-fig. 2). The above facts show conclusively that the halves of the bivalent chromosomes arranged at the equator of the spindle represent the conjugated univalent chromosomes. Therefore the first division is a reducing division.

## X. Summary.

1. The resting nucleus of the spermatogonium contains a large nucleolus and several small chromatin masses.
2. In the metaphase plates of the spermatogonia all the chromosomes are divided at the same time. In this stage it was not possible to count the chromosomes accurately, but many symmetrical pairs of the same were easily distinguished.
3. The resting nucleus of the primary spermatocyte contains a large chromatic nucleolus.
4. The conjugation of the chromatin threads takes place by parasympapsis.
5. The chromosome nucleolus presents itself throughout the synapsis and the growth stages.
6. In the primary spermatocyte the idiozome is conspicuously present.
7. The number of chromosomes in the first division is nineteen, namely : eighteen bivalent and one accessory.
8. The first division is reducing and heterotypic. The accessory chromosome now passes undivided to one pole, thus producing two groups of spermatocytes, one with and the other without the accessory chromosome.

9. The resting stage of the secondary spermatocytes can rarely be observed, which makes it probable that this stage is of very brief duration.
10. The second pairing of the chromosomes in the second division was not observed.
11. The second division is equal and homotypic. The accessory chromosome divides like the ordinary ones.
12. The behavior of the centrosome in the development of the spermatozoa is almost similar to the condition found by MEVES in man.
13. The chromatoid corpuscle makes its appearance during the growth stage. In the final development of the spermatozoa it is probably cast off, out of the body of the spermatozoa.
14. The mitochondria make their appearance during the postsynaptic stage. In the spermatids most of them give rise to a mass which is similar to the "Nebenkern" in the insects, while the main portion finally comes to occupy the middle part of the spermatozoon.

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#### EXPLANATION OF FIGURES.

All figures were drawn with the aid of a camera lucida, using a Zeiss 1/12 objective and a compensating ocular 12, except Figs. 1-4 which were drawn with a Zeiss 5 ocular and Figs. 5, 6 with a Zeiss 4 ocular. The following abbreviations have been employed:

- |                |                        |                |                        |
|----------------|------------------------|----------------|------------------------|
| <i>a. ch</i> , | —accessory chromosome. | <i>ch. c</i> , | —chromatoid corpuscle. |
| <i>i. c</i> ,  | —interstitial cell.    | <i>mt</i> ,    | —mitochondria.         |
| <i>s. sc</i> , | —Sertoli-cell.         |                |                        |

#### PLATE XI.

- Figs. 1-3. Section of the testis of a young animal.
- Fig. 4. Metaphase plates of primary spermatogonia.
- Figs. 5, 6. Section of the testis of an adult animal (3 years old).
- Figs. 7, 8. Primary spermatogonia in resting stage.
- Fig. 9. Prophase of a primary spermatogonium.
- Fig. 10. Polar view of a metaphase plate of a primary spermatogonium.
- Fig. 11. Lateral view of metaphase plate of a secondary spermatogonium.
- Fig. 12. Anaphase of a secondary spermatogonium.
- Figs. 13-15. Resting primary spermatocytes.
- Fig. 16. Presynaptic stage (leptotene stage).
- Figs. 17, 18. Synapsis, showing parallel arrangement of chromatin threads.
- Fig. 19. Postsynaptic stage; complete fusion occurs between the pairing threads.
- Fig. 20. Postsynaptic stage.
- Figs. 21, 22. Spermatocytes in late postsynaptic stage.

#### PLATE XII.

- Figs. 23, 24. Spermatocytes in late postsynaptic stage, showing mitochondrial granules.
- Figs. 25-27. Successively later postsynaptic stages.
- Figs. 28-30. Prophases of first reduction division.
- Fig. 31. Polar view of metaphase of first division.

Figs. 32, 33. Polar views of metaphase plates of first division, showing eighteen chromosomes.

Fig. 34. Polar view of metaphase of first division, showing 16 chromosomes.

Figs. 35, 36. Polar views of metaphase plates, of first division, showing nineteen chromosomes.

Figs. 37, 38, 39, 43. Lateral views of first division, showing the accessory chromosome.

Figs. 40—42. Side views of first division; long axis of chromosomes is at right angle to that of spindle.

Figs. 44, 45. Lateral views of anaphases of first division.

#### PLATE XIII.

Fig. 46. Telophase of first division.

Figs. 47—50. Secondary spermatocytes in resting stage, showing chromatoid corpuscle, mitochondria and chromatin nucleolus.

Fig. 51. Resting secondary spermatocyte and metaphase plate of second division.

Figs. 52—54. Polar views of metaphase plates of second division.

Fig. 55. Side view of metaphase plate of second division.

Fig. 56. Side view of metaphase plate of second division, showing mitochondria.

Fig. 57. Anaphase of second division, showing chromatoid corpuscle.

Figs. 58, 59. Telophases of second division.

Figs. 60, 62. Spermatids, showing mitochondrial granules.

Figs. 61, 63, 64, 65. Spermatids, showing chromatoid corpuscle and centrosome.

Fig. 66. A spermatid, showing archoplasm.

Fig. 67. A spermatid, showing centrosomes.

Fig. 69. A spermatid, showing mitochondria.

Figs. 68, 70, 71, 72, 73. Development of Spermatozoa.

Figs. 74, 75. Mature spermatozoa.

# The Spermatogenesis of Domestic Mammals.

## II. The Spermatogenesis of Cattle (*Bos taurus*).

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With Plates XIV-XVI and one Text-Figure.

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The present paper deals with the spermatogenesis of cattle which most probably is *Bos taurus*. Before going further it is perhaps advisable to state briefly the points of special interest revealed by this investigation which can be enumerated as follows:—1. In the testes of embryos and quite young animals amitotic division of spermatogonia frequently occurs. 2. One accessory chromosome is present which is quite similar in its behavior and form to that of the horse. 3. The behavior of the centrosome in the development of the spermatozoa differs considerably from that observed in the horse. 4. No evidence of the occurrence of parasympapsis as shown in the horse, is to be found, but accurate observation of the growth period brings us to the conclusion that telosympapsis probably occurs in the synaptic period of the cattle. 5. The chromosomes thus united by telosympapsis are separated in the first reduction division.

The materials used in this study were chiefly obtained during the summer and autumn of 1917 at the slaughter house at Minowa in Tôkyô.

The testes which were removed from the scrotum were immediately cut into very small pieces and transferred into fixing agents. For the fixation FLEMMING's chromo-aceto-osmic acidmixture (strong formula), CHAMPY's fluid BOUINS picro-formol and CARNOY's fluid (acetic alcohol

with sublimate) were chiefly used, but only the former two gave satisfactory results.

The sections were cut 5—10 $\mu$  in thickness. For the staining HEIDENHAIN's iron-haematoxylin and FLEMMING's triple stain were used, and for the staining of the nucleolus ZIMMERMANN's method (iodine green, fuchsin). Smeared preparations were also used which were stained with the same stains as those for the sections.

In the testes of adult animals various stages of germ cells could be seen in a single section, but mitotic figures were rarely found. Therefore for the studies of the reduction division and the mitosis of the spermatogonia, testes of young individuals were chiefly used. In quite young animals amitotic divisions of the spermatogonia frequently occur while the mitotic divisions are rare.

The author wishes to express his hearty thanks to Prof. ISHIKAWA to whom he is greatly indebted for much valuable advice given him during the study. Thanks are also due to Prof. YATSU of the Science College who kindly lent the necessary literature on the subject.

### I. The Amitotic Division of the Spermatogonia.

The amitotic division of the spermatogonia in the earliest generation is very frequently found in the testes of embryos and of quite young animals (Fig. 2). In the testes of such animals, some of the cells of the wall of the tubule are seen to grow rapidly in size, becoming about two or three times as large as they were at the beginning of this stage (Figs. 1, 3). Subsequently they fall into the lumen of the tubule (Fig. 1). Their nuclear organization and their cytoplasmic structure are quite similar to those of the spermatogonia found in the testes of adult animals (Figs. 1, 2, 31, 32). The nucleus is relatively large compared with the amount of cytoplasm, and the chromatin granules are scattered throughout the nucleus (Figs. 2, 4). The nucleoli usually appear round in shape, in most cases they number only one but sometimes two or three (Figs. 4 to 6). These cells are the spermatogonia in the earliest generation.

The cell increases steadily in size and the shape of the nucleus becomes

irregular (Fig. 8). When the cells have thus reached, or are about to reach the maximum size (about twice as large as the spermatogonia in the earliest generation), the nuclear wall begins to constrict at one side where a cytoplasmic mass is always situated (Figs. 9, 10). This cytoplasmic mass is apparently similar to that described by VON RATH ('93) in the salamander. Soon a cleft appears at this constricted point (Fig. 10) which continues to deepen, until a complete division of the cell is effected (Figs. 11—14). The same type of amitotic division was reported by McGREGOR ('99) in the spermatogonia of *Amphiuma*, but PATTERSON ('08) has established two types of amitotic division in the pigeon's egg; in one type the nucleus elongates, after which a constriction in the nuclear membrane appears on the entire circumference of the nucleus; in the other type a nuclear plate is laid across the nucleus and the division consists in a splitting of this plate. He added that besides these the modified form of the first type occurs. In this type the constriction of the nuclear wall proceeds from one side, and the nucleus usually does not elongate previous to the appearance of the constriction.

The method of amitotic division in cattle is quite similar to this modified form.

At the early stage of this division, the cytoplasmic granules are gathered together and appear as a granulated mass, which comes to lie on the concave side of the nucleus (Figs. 9, 10). Sometimes, however, the mass surrounds the nucleus more or less completely. When a trace of fission has appeared on the nuclear wall, this granular mass draws together to form a rounded sphere in which no other substance is to be seen (Figs. 10, 12). The centrosphere of ring form such as that described by MEVES ('91) in salamander, can not be found, but the form and behavior of the mass is quite comparable to the sphere described by VON RATH ('95). After the nucleus has divided into two, the sphere again begins to disintegrate into the granules and is placed between the two daughter nuclei (Figs. 14, 16). From the behavior and the position of the sphere, it seems more probable that the sphere might play an important rôle in the amitotic division, independent of centrosomes. A view similar to this was put forward by MEVES ('91) in the amitotic division of the spermatogonial cells of Salamander, where he says: "Betrachtet man dagegen ihre Beteiligung bei demselben Vorgang in den

Spermatogonien, so scheint es kaum möglich, den Gedanken abzuweisen, daß die ringförmige Sphäre einen mechanischen Einfluß auf die Kerntheilung ausübt."

The amitotic division usually begins in the fission of the nucleolus (Figs. 9, 12), being followed by that of the nucleus, while in some cases the nucleolus does not seem to divide (Figs. 8, 10). The nucleus seldom divides into three or more at the beginning of this division (Fig. 7).

During the amitotic division the nucleus usually remains in the resting state (Figs. 8—14), and there is no formation of spiremes or chromosomes. But some of the spermatogonial cells in which the spiremes have been formed, are seen suddenly to increase in size and begin to divide amitotically (Fig. 18). In such a case the spiremes gradually disappear, and the nucleus again enters into the resting state (Fig. 16).

As the amitotic nuclear division is not followed by the division of the cell body, multinuclear cells are formed by it (Fig. 17). The nucleus thus formed by amitosis usually appears irregular in form and contains a small amount of chromatin granules which are scattered throughout the nucleus. Their nuclear organization is quite similar to that of the Sertoli-cells.

## II. The Spermatogonia.

*Spermatogonia in the earliest generation* :—Only a few spermatogonial cells of the earliest generation are found in the testes of quite young animals. As stated above, some of the spermatogonial cells are divided by amitosis, while the others are multiplied by mitosis, thus two kinds of cells are produced by the two methods of cell division. In the cells produced by mitosis, the nuclei are of large size, and numerous chromatin granules are scattered throughout the nucleus. These cells are the spermatogonia in the earliest generation.

The number of chromatin granules gradually increases, which afterward arrange themselves along fine threads in an entangled mass around the nucleolus (Figs. 22, 23). Then the chromatin spiremes are formed which are distributed throughout the whole space of the nucleus, meanwhile the nucleolus begins to disappear (Fig. 22) or to break into small pieces (Fig. 20), leaving a plastin remnant. The spiremes now become gradually denser and thicker,

until the granular appearance is entirely lost, and the cell enters into a prophase stage. Longitudinal splittings of the chromosomes thus formed now become clearly visible (Fig. 24), and can be seen till the late prophase (Fig. 25). It is difficult to determine whether the spireme is not continuous, as stated above, but it is very probable that it is so, as the end of each chromosome can be easily distinguished throughout the entire stage (Fig. 22—24). The spiremes continue to become shorter and thicker, until they assume short rod shapes (Fig. 25). Sometimes the nucleoli still remain among the chromosomes in the late prophase. It is, however, difficult to distinguish them in the preparations stained with iron-haematoxylin, while they can clearly be seen in those stained with ZIMMERMANN'S method.

In the polar view of the equatorial plate of the metaphase the number of chromosomes may be counted as thirty three, most of which are arranged radially around a central space (Fig. 28).

As many authors have already pointed out in insects and other animals, the chromosomes of the spermatogonia in every stage vary considerably in size and form (Figs. 28, 29, 37, 38). In the diploid groups, as shown in Figs. 29 and 38, the chromosomes are found to correspond two by two. These can clearly be seen in the metaphase plate of such cells as shown in Figs. 28, 37. Among the chromosomes of these cells the three largest ones can easily be distinguished. From the size and form of these chromosomes, it is evident that one of them has no mate, and this odd one is evidently an accessory chromosome. Every chromosome now simultaneously divides into two portions along the longitudinal split already described in the spireme stage, no special chromosome with different behavior being seen among them (Figs. 26, 27), although according to WODSEDALEK ('14) the accessory chromosome in the horse, as a rule, divides a little in advance of the ordinary ones.

At the telophase the chromosomes are not fused, but are so closely appose to each other that they can not be clearly distinguished. Later on the nuclear wall reappears, and the chromosomes become separated from each other (Fig. 30), their individuality thus becoming again clearly distinguishable. No chromosome of this stage shows any longitudinal splitting (Fig. 30).

*Spermatogonia in adult animals:*—As in the horse, in the testes of adult

animals two generations of spermatogonia can be distinguished, a penultimate and an ultimate. But as several generations of spermatogonia are found in one and the same testis this distinction is not quite positive. It is, however, convenient to distinguish two generations and to use MONTGOMERY's terms "penultimate" and "ultimate" instead of "primary" and "secondary," used by various authors. MONTGOMERY's ('11) view in using these terms in his study in *Euschistus* is as follows: "In the testes of adult individuals are found two generations of spermatogonia which it will be convenient to call the 'penultimate,' and 'ultimate,' these terms being preferable to 'primary' and 'secondary' of most writers, for the reason that primary and secondary employed in the strict sense should refer to the first two generations of the germinal cycle. Whether there are three generations of them in adults was not positively ascertained."

The resting nucleus of both spermatogonial generations usually contains one large nucleolus and many small chromatin masses (Figs. 31, 32, 40). The nucleolus of the penultimate spermatogonia is more or less larger than that of the spermatogonia in the earliest generation (Figs. 4, 6, 31). It gives an appearance of granular construction composed of aggregated chromatin granules. These chromatin granules as well as those in the linin later begin to arrange themselves along fine threads (Figs. 33, 34). At the commencement of the prophase the nucleolus gradually disappears, leaving a plastin remnant behind (Figs. 33, 34). A similar condition has been found by JORDAN ('11) in opossum, where the function of the nucleolus is stated to favour the proposition that it plays "a part as a store-house of chromatin, which contributes at mitosis to the formation of chromosomes."

In the resting period of all the spermatogonial cells (in both adult and young animals) (Figs. 2, 31, 32), beside the large nucleolus, one rod shaped chromatin mass usually appears. It is difficult to determine whether this mass represents the future accessory chromosome or not, as it later disappears, and it is impossible to trace this chromatin mass during the metakinesis. In the resting period of the spermatogonia the chromatin nucleoli were found by WODSEDALEK ('13) in pig, and by BACHHUBER ('16) in rabbit, both authors holding the view that the nucleoli and the accessory chromosomes are one and the same structure. In the prophase and the

metaphase the behavior of the chromosomes is just the same as that of the spermatogonia in the earliest generation, but the size of the chromosomes of the penultimate spermatogonia is larger than that of the earliest generation (Figs. 37, 38, 29). Fig. 37 exhibits a polar view of the metaphase of the penultimate spermatogonium. The chromosomes here are arranged radially around the central space, and their number and form are just the same as those of the spermatogonia in the earliest generation. The idiozone is situated in close contact to the nuclear wall, but a centrosome can not be demonstrated within it.

### III. The Spermatocyte.

#### A. THE GROWTH PERIOD.

*The leptotene stage* :—The daughter cells resulting from the division of the ultimate spermatogonia are the spermatocytes, and these pass gradually to the growth period.

The chromosomes are not fused immediately after the division of the ultimate spermatogonia thus it is possible to distinguish individual chromosomes (Figs. 39, 46, 47). The next change is that the nucleus becomes larger, while the chromosomes grow more irregular in form (Fig. 47), and no nucleolus is formed in any part of the nucleus.

It is conceivable that in this stage a continuous chromatin spireme is not formed, as free ends of certain spiremes may always be distinguished (Figs. 48—50). The cytoplasm appears granular, but its precise structure as well as the idiozone and the centrosome can not be identified (Figs. 39 to 45).

*The synaptene stage* :—The leptotene threads begin to converge towards one side of the nucleus, leaving a clear space on the other side (Figs. 41, 51). Neither an idiozone nor a centrosome can be identified at the converging point of the chromosomes, although in the horse the idiozone is situated at this point. Finally the spiremes collect together and form a mass, but there is no evidence that the chromosomes become fused and thus lose their individuality (Fig. 51). All the appearances of the nuclear structures indicate rather that the spiremes are only very closely massed, for the sections always

show the outlines of individual spiremes distinctly (Figs. 41, 51). In the synapsis the nuclear wall expands rapidly and soon apparently disintegrates (Figs. 41, 42, 51, 52).

The spiremes arrange themselves in a very much tangled mass of loops but indications of their parallel arrangement as in the horse do not appear. In Fig. 41 some spiremes appear to form such an arrangement, which is, however, to be looked upon as an incidental phenomenon, as the ends of such spiremes very often lie apart from each other.

*The pachytene stage* :—Near the end of the synaptene stage the whole mass of the spiremes begin to move toward the center of the nucleus. At this period the spiremes appear about half the original number and fully twice as thick as those of the leptotene stage, while the bulk of the cell increases rapidly (Fig. 52). The movements of the chromatin spiremes are carried out further, until they fill up the nucleus thoroughly, while the nuclear wall becomes spherical and is more clearly defined (Fig. 44).

*The diplotene stage* :—Following upon the pachytene stage comes the diplotene. At the beginning of this stage the cells increase in size, becoming twice as large as the telophase of the ultimate spermatogonia, and the spiremes stain faintly with iron-haematoxylin, showing a granular appearance (Figs. 44, 54) which is similar to that described by Gross\* ('07) in *Pyrrhocoris apterus L.* The spiremes are too convoluted to show their exact number, but it is most probable that they are of haploid number (Figs. 44, 45, 54). At the beginning of this stage the longitudinal split of chromosomes is rarely to be seen.

Together with the growth of the cell the granulated spiremes gradually become thicker, increase their staining capacity and show distinct evidence of longitudinal splits (Figs. 54, 55). The careful observation of the individual spireme shows that the spireme consists of large chromatin granules, and in its middle points a transverse constriction usually appears (Figs. 55, 56). But whether the constriction represents the conjugated point of two spiremes, or whether the condition is purely incidental, can not be determined. The

\* Gross ('07) found that in *Pyrrhocoris* the chromosome consists of "Microsomen" where he states: "Die Zahl der Microsomen scheint bei den größern Elementen ungefähr 8—10 zu betragen. Ob die Zahl aber bei allen Chromosomen dieselbe ist, wage ich nicht zu entscheiden."

constriction can, however, be traced during the whole diplotene stage (Figs. 54—57).

Later on the spiremes shorten and become condensed, assuming straight or bent rods; the surface of each chromosome remains, however, rough and somewhat filamentous, until they arrange themselves in the metaphase plate (Figs. 57, 63). As in the case of the horse, the rings or loops of the chromosomes do not appear in the late prophase, but in strongly decolourized preparations the longitudinal split of the chromosomes appears very distinctly (Figs. 56, 57). During the prophase, it is impossible to count the number of chromosomes accurately, since they never lie in a plane but overlie one another (Figs. 55—57).

*The chromosome nucleolus* :—In the beginning of the leptotene stage one of the chromosomes does not grow to a fine chromatin filament but remains in its original form, while the others develop to the leptotene threads. During the synapsis it can not be recognized, which is to be accounted for by the fact that it usually takes its position within the entangled mass of the chromatin spiremes.

While the ordinary chromosomes now become less compact and stain more faintly, this chromosome retains its staining capacity and its sharp contour (Figs. 43, 53). From this stage (pachytene stage) until the late prophase it usually remains in contact with the nuclear wall and generally appears oval, varies in size and form, sometimes heart shaped, occasionally bipartited (Figs. 53—55). During the prophase it stains like the chromatin spiremes with ZIMMERMANN's method. Its behavior and its form correspond almost exactly with the accessory chromosome or the chromosome nucleolus of the horse.

In the late prophase, when the ordinary chromosomes have assumed rod shape, the chromosome nucleolus is situated within the ordinary chromosomes, but it can be identified by its shape and behavior.

*The cytoplasmic structure* :—The cytoplasmic structure, with the exception of the idiozome and the mitochondria, can not be identified, but in the horse one or two small chromatoid corpuscles make their appearance during the growth period.

In the early diplotene stage when the spiremes are spread throughout the

nucleus, a cytoplasmic body very faintly stained with iron-haematoxylin can be seen on careful observation (Figs. 43—45). It is represented only as an oval or a round mass. This body may be the idiozome, but the centrosome can not be found within it (Figs. 44, 45). It is impossible to assert whether the absence of the centrosome in the idiozome is due simply to its small size, or that the former has no connection with the latter. It seems to me, however, that the latter view is more probable. The same view is held by MACHIDA ('17) in the spermatocyte of a grasshopper, *Atractomorpha*. The nuclear wall as well as the idiozome began to disappear, and the division of the idiozome could not be observed. In the horse, however, at the late prophase the idiozome is divided into two spheric bodies which gradually move apart from each other.

In the growth stage the mitochondrial granules appear abundantly in the cytoplasm, being scattered throughout the cell body (Figs. 61, 62).

#### B. THE REDUCTION DIVISION.

*The ordinary chromosomes in the first reduction division* :—At the commencement of the metaphase when the chromosomes assume short rod shape, both the longitudinal split and the transverse constriction of the chromosomes become faintly visible (Figs. 63—65). In good polar view of the equatorial plate of the metaphase the number of chromosomes is always counted to be seventeen which represents the haploid number of chromosomes in the spermatogonia. In the horse two kinds of metaphase plates are distinguishable, namely, in one half of the metaphase plates eighteen chromosomes are represented and in the other half nineteen. The chromosomes in this stage show distinct and constant differences in size (Figs. 63—65). Even though the difference in size between neighbouring chromosomes is very little, five large sized, five small sized and two very small sized ones can be distinguished (Figs. 63—65).

In the strongly decolourized preparations the side view of the metaphase of the first division shows that sixteen bivalent chromosomes are arranged in the equatorial plate with their longitudinal split parallel to the axis of the spindle, and thus the transverse constriction of the chromosomes are at right angles to the same (Fig. 67). The transverse constriction becomes more and

more conspicuous, and the chromosomes consequently assume dumb-bell shape. In this period the longitudinal splits are still recognizable, though faintly, in the fairly well stained preparations (Fig. 67). Finally the chromosome becomes separated into its components, as shown in Figs. 69 and 70. From the behavior of the chromosome so far studied it is evident that in the first division the chromosome is divided along the constriction which appeared first at the prophase. If the constricted point of the chromosome represents the conjugated ends of the univalent chromosomes, then this division must be looked upon as a reducing division. Details as to the conjugation and the reduction of the chromosomes will be discussed later on in the general consideration.

When the chromosomes thus separated move toward the respective pole, **U**-or **V**-shaped ones, which are usually observed in many other animals, are not produced (Figs. 69, 70), they simply assume the short rod shape, and their longitudinal splits entirely disappear (Fig. 70). During the metaphase and the anaphase the behavior of the ordinary chromosomes is entirely similar to that of the chromosomes of the horse.

As soon as the chromosomes arrive at the poles, they aggregate so closely that the individual chromosomes, with the exception of the accessory, are scarcely distinguishable, but in fairly good stained preparations most of the chromosomes can sometimes be made out (Fig. 70). Later the chromosomes become more and more shortened, but their individuality is still recognizable. In the telophase all the chromosomes collect closely together and thus their individual outline becomes entirely lost to view (Figs. 71, 72).

*The ordinary chromosomes in the second reduction division:*—As the second division often appears in contact with the first division, it is conceivable that in most cases the telophase of the first division, without the previous resting period, passes immediately to the second division. In this division all the chromosomes arrange themselves in the equatorial plate at the same time (Figs. 75—77) where they show a tendency to gather into a mass, which makes it difficult to count them with certainty. In fairly good stained preparations the number of chromosomes is, however, estimated to be sixteen or seventeen (Figs. 75, 76). Whether this indicates the true number of chromosomes in the second division can not be positively stated, since only

a few metaphase plates were observed. Each of the seventeen elements is univalent instead of bivalent, and often shows a longitudinal split. In those cells in which seventeen chromosomes are counted, one large chromosome usually appears which is quite similar in shape to the accessory chromosome of the first division (Fig. 76), and very likely is the same.

Sometimes, as in the case of the horse, incomplete fusion of the chromosomes is seen to occur, and in such a case occasionally nine or ten chromosomes are found. From the appearance of the chromosomes, it is evident that their fusion in the second division is an incidental phenomenon.

In the equator of this division each of the seventeen chromosomes becomes so placed that the line of the longitudinal split coincides with the equatorial plane and along this line all the chromosomes are divided at the same time (Figs. 77, 78). From this fact it is certain that the real character of the second division is simply an equation division, the accessory chromosome being also divided into two, like the ordinary ones (Figs. 77, 78). The chromosomes in the anaphase and in the telophase are never fused but maintain their individuality (Figs. 79—82).

*The accessory chromosome*:- In the beginning of the first division when the ordinary chromosomes become arranged at the equatorial plate, the accessory also lies for a while in the same plate with the ordinary ones (Fig. 66). In the polar view of the metaphase plate the accessory chromosome can not with any certainty be distinguished from the ordinary, but in the later stage it is easily possible to make out this chromosome since its behavior and its form are different from those of the ordinary ones. In the earlier metaphase, when the chromosomes assume short rod shape, all the cells contain seventeen chromosomes and in the side view of the same no special chromosome can be seen (Figs. 58, 66). In the later stage when the chromosomes begin to divide, assuming dumb-bell shape, the accessory passes undivided to one pole of the spindle in advance of the ordinary chromosomes (Figs. 68, 69). This shows that the accessory remains for a time in the metaphase plate.

The accessory chromosome can be distinguished even at the late anaphase (Fig. 70), but in the telophase when the chromosomes collect so closely together that their individuality becomes entirely lost to view, the accessory

also becomes indistinguishable. The behavior and the form of this chromosome (accessory) during the anaphase and the telophase of the first division are quite similar to those of the accessory chromosome of the horse. From what is stated above it is obvious that one half of the daughter cells thus formed contains the accessory, while the other half does not.

In the second reduction division, as stated above, the accessory chromosome can be seen in one half of the metaphase plates and is divided at the same time with the ordinary chromosomes. It can be recognized till the telophase of the second division, and thus we can distinguish two kinds of cells, namely, approximately one half of the cells contains sixteen chromosomes and the other half seventeen (sixteen ordinary and one accessory chromosome) (Figs. 79—82).

*The resting stage of the secondary spermatocyte:*—As in the case of the horse, the resting stage of the secondary spermatocyte is rarely found in cattle (Fig. 60).

The nuclei of the resting secondary spermatocytes usually contain several chromatin masses (Karyosomes) (Figs. 60, 73, 74). The difference in their size is very small, no special large chromatin nucleolus like that which we find in one half of the nuclei of the same stage in the horse can here be found. The dimorphism of the cells occurring in this stage of the horse can also not be recognized in cattle (Fig. 60). Both the idiozome and the mitochondrial granules are found in this stage, a large portion of the latter being placed near the former (Figs. 73, 74).

*The cytoplasmic structure:*—During the reduction division the mitochondria lie outside the spindle (Fig. 66). When the cell is divided into two, they seem to be equally distributed in the cytoplasm of the daughter cells (Fig. 71).

A small spheric body stained faintly with iron-haematoxylin sometimes, though rarely, appears in the cytoplasm of the cells in the reduction divisions, situated close to the cell membrane (Figs. 65, 75). It is difficult to determine whether this body corresponds to the chromatoid corpuscle of the horse or not, for in succeeding stages this body is not to be found. It can not be compared with the chromatoid corpuscles of the horse, where they first make their appearance during the growth stage and are always found in all the following stages till the development of the spermatozoa. From its behavior

and appearance it is also probably not to be compared with the chromatoid corpuscle described by WILSON ('13) in *Pentatomidae*.

#### IV. The Spermatids up to the Formation of the Spermatozoa.

After the second division the chromosomes gradually begin to disintegrate into minute granules and later become scattered throughout the nucleus (Figs. 83, 84). The nuclei of the spermatids in the resting stage usually contain two or three large chromatin nucleoli (karyosomes) and several small chromatin masses (Figs. 86—88). Thus the dimorphism as regards the existence of the chromatin nucleolus, can not here be recognized (Figs. 85—89). STEVENS ('11) in guinea-pig and JORDAN ('11) in opossum also state that the spermatids and the spermatozoa are not visibly dimorphic.

During the resting stage of the spermatid the centrosome appears near the nuclear wall and is surrounded by a clear area (Figs. 87, 89, 90).

In this stage the idiozome distinctly appears in close contact with the nuclear wall, and shows a clear contour and compact structure (Figs. 86, 88, 89). Most of the mitochondria usually lie near the centrosome (Fig. 85).

In a later stage two centrosomes become clearly visible. They lie side by side, and are connected by a thick filament (Figs. 87, 89). These two centrosomes are probably produced by division of the original one. With this change of the centrosome the chromatin materials begin to collect on the surface of the nucleus, leaving only one or two chromatin masses (karyosomes) (Figs. 88—94). Simultaneously with this, the nucleus gradually shifts its position to one side of the cell which is destined to become the anterior end of the spermatozoon, while a large amount of the cytoplasm with the mitochondrial granules gathers together at the posterior part of cell body (Figs. 91, 93).

The change of the centrosome during the formation of the spermatozoa is considerably different from that observed in the horse. The two centrosomes gradually part from each other, assuming an elongated dumb-bell shape, and change their relative position in such a way that the axis of the dumb-bell comes to be situated perpendicular to the surface of the nucleus (Figs. 93,

94). The two centrosomes can thus be distinguished as a proximal and a distal, the former at the same time comes to be placed close to the nuclear wall (Fig. 93). Meanwhile a fine filament is seen to proceed from the distal centrosome backward towards the surface of the cell, which later becomes the axial filament of the spermatozoon (Figs. 93, 94). This seems to indicate that the axial filament is mainly developed from the distal centrosome which corresponds to the outer centrosome in the horse.

The idiozone which has appeared in the resting spermatids, gradually diminishes in size, becoming more and more homogeneous (Figs. 86, 88, 89). When the nucleus begins to migrate towards one side of the cell, the idiozone which remains close to the nuclear wall, moves to a point directly opposite to the centrosome in contact with the nuclear wall (Figs. 88, 89). From these phenomena it is evident that the archosome is formed from the idiozone as indicated by MEVES.

Simultaneously with the migration of the idiozone, a slight depression can be detected on the anterior part of the nuclear wall (Fig. 92). The archosome finally becomes fixed as a small spheric body at this point, just as WODSEDALEK ('13) has shown to be the case in the pig.

In the daughter cells of the secondary spermatocyte the mitochondria still lie scattered in the cell body. Afterwards they commence to gather around the centrosome (Fig. 91). In the horse, the main portion of the mitochondria in the final development of the spermatozoa fuse and form a mass which is similar to a "Nebenkern" in insects. During the final transformation of the spermatid a portion of the mitochondria is located at the posterior part of the cell where it encloses the axial filament and the centrosome (Figs. 91, 93, 95), the rest of them are probably cast off, out of the body of the spermatozoa (Fig. 95). In the mature spermatozoa the mitochondria come to occupy the middle part of the spermatozoon (Fig. 98).

Most of the chromatic material now collects increasingly at the peripheral part of the nucleus (Figs. 88—92). In the development of the spermatozoa neither the chromatin nucleolus nor the true nucleolus (plasmosome) can be seen (Figs. 85—94). According to JORDAN ('11), in opossum a conspicuous central plasmosome appears during the transformation of the spermatid.

With the changes of the nucleus, the tail becomes more and more

elongated, and a small portion of the cytoplasm finally comes in contact with the axial filament.

In the mature spermatozoa a very small amount of cytoplasm is used to envelope only about half or a third of the length of the axial filament, the rest of the cytoplasm is probably cast off out of the spermatozoa (Figs. 95, 96, 98).

In the final development of spermatozoa a dense spherical cytoplasmic mass, similar in appearance to the idiozome, becomes frequently visible, which from the close similarity of its appearance and structure to that of the archoplasm, is probably to be regarded as a portion of the idiozome. This body is probably cast off out of the spermatozoa together with the cytoplasm.

## V. General Considerations.

### A. AMITOTIC DIVISION.

Amitosis or direct division in the germ cell of the higher animals is in reality a rare and exceptional process. In the germ cells of *amphibia* it is described by LA VELLETE ST. GEORGE ('85), MEVES ('91), VON RATH ('91, '93), BENDA ('93) and McGREGOR ('99); in insects by PREUSSUE ('95), GROSS ('01) FOOT and STROBELL ('11) and WIEMAN ('10). CHILD ('07) found amitosis occurring in the germ cells of certain *Cestoda* and *Annulata*.

As to the meaning of amitosis MEVES ('91, '93) believed that in salamandra amitosis is a normal process in the spermatogenetic cycle and stated that the daughter cells (spermatogonia) produced by amitosis likewise come to divide mitotically, giving rise eventually to functional spermatozoa. On the other hand VON RATH ('91) asserted that in *Astacus* and other animals the cells divided by amitosis do not belong into the developmental cycle, but are destined to degenerate, and to be used as nutritive material by the normally developing sex cells. McGREGOR ('99) in *Amphiuma* supported MEVES's interpretation. CHILD's ('07) observation on the germ cells of *Monizia* is as follows: the early division of germ cells is amitotic, then comes a growth period at the beginning of which a spireme is formed, and subsequently they (the cells produced by amitotic division) divide mitotically during maturation divisions.

In cattle, as stated above, an irregular outline of nuclei and a small

amount of chromatin granules are characteristic of the nuclei of daughter cells which are produced by amitosis, while the nuclei of the spermatogonial cells are usually spherical and contain numerous chromatin granules. No case was observed where a cell divides mitotically which has once been divided by amitosis.

The above data show conclusively that the cells divided by amitosis do not develop to sex cells, but probably degenerate and are used as nutritive materials by the germ cells as vom RATH asserts, where he says: "Faßt man nun in kurzem die Resultate meiner Untersuchungen bei *Astacus* mit den von Platner und Hermann bei den Pulmonaten, der Maus und dem Salamander gewonnenen Beobachtungen zusammen, so glaube ich zu folgender Schlußfolgerung berechtigt zu sein: In allen Fällen, in welchen eine amitotische Kernteilung im Hoden beobachtet wird, vollzieht sich diese Kernteilung nur an den Randzellen (Stützzellen). Letztere stehen weder mit der eigentlichen Spermatogenese noch mit den Regenerationserscheinungen in direkter Beziehung. Die Samenbildung kommt nur auf mitotischem Wege zu Stande und ebenso die Regeneration. Eine Umwandlung von Randzellen (Stützzellen) zu Spermatogonien findet nicht statt. Demnach bildet die amitotische Kernteilung im Hoden hinsichtlich ihrer biologischen Bedeutung keine Ausnahme mehr und steht einer einheitlichen Auffassung der amitotischen Kernteilung nicht mehr im Wege."

As many authors have already pointed out, it is more probable that amitosis is the result of special nutrition by which the spermatogonial cells are affected. The following facts may be cited as favorable to this view: 1. As stated above the nuclei of the spermatogonia which begin to divide amitotically, are always of large size. 2. Sometimes the prophase nuclei in which the spiremes are clearly seen, rapidly increase in size, becoming about twice as large as those of the spermatogonial cells, and finally come to be divided by amitosis. 3. In the testes of quite young animals amitosis occurs more frequently than mitosis.

PATTERSON ('08) has found amitotic division in the pigeon's egg and believed that amitosis is the result of special physiological conditions which create a stimulus to cell-division where he says: "Just what these conditions are, we are of course unable to say, but whatever factors are involved in

bringing about the rapid growth of any region would seem to be concerned in coming amitosis."

A view different from those of PATTERSON and mine is maintained by Wieman ('10 b) who studied the amitotic division of germ-cells in *Leptinotarsa signaticollis*. He says: "What causes the change from mitosis to amitosis? It has appeared to me that here likewise a gradual diminution in nutrition is responsible. It might be assumed that the object of the long rest stage or growth period in the development of the ovum is to elaborate food and formative material for maturation, fertilization and embryonic development. We might further assume that the same process provided for a certain number of mitotic divisions extending through the early cleavage. The direct method of cell division sets in because of a deficiency in the amount of nutritive material (oxygen?) necessary for continued mitotic divisions. This is in keeping with the fact that amitosis occurs usually under abnormal metabolic conditions which are unfavorable to normal metabolic processes. Amitosis might be regarded as a simpler form of cell division, not so much because it takes place in the absence of spindle and chromosomes, as for the reason that it can occur under circumstances that makes mitosis impossible."

#### B. THE SYNAPSIS AND THE REDUCTION.

As far as I am aware, there are comparatively few complete accounts of the synapsis and the reduction of chromosomes in any mammal at the present time. The researches of MEVES ('98), GUYER ('10), STEVENS ('11), WODSEDALEK ('13, '14) and others have chiefly dealt with individual sections of the process, with the origin of the archosome; the history of the centrosomes in relation to the spermatozoa; and the behavior of the accessory chromosomes. Only WINIWARTER ('01) and JORDAN ('11) demonstrated the subjects accurately.

JORDAN ('11) in opossum found that during the synizesis the first numerical reduction of the diploid group of chromosomes occurs apparently by telosynapsis, where he states: "The character of the early prophase chromosomes (double threads; later loops and paired threads) suggests that perhaps no sharp distinction really exists between the end-to-end and side-to-side method of conjugation. Here the one appears to follow the other. The reduction takes place first by end-to-end conjugation. Later on, the limbs of

the loops approximate and fuse more or less completely. In this condition the bivalent chromosome is divided in metakinesis."

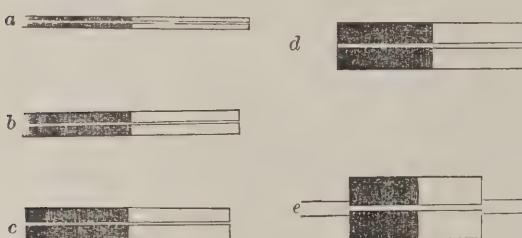
On the question of synapsis and reduction in *Hemiptera Tomopteris*, *Bastracoseps* and some other forms WILSON ('12) has published an excellent and critical consideration in which he stated that: "The cytological problem of synapsis and reduction involves four principal questions as follows: (1) Is synapsis a fact? Do the chromatin-elements actually conjugate or otherwise become associated two by two? (2) Admitting the fact of synapsis, are the conjugating elements chromosomes, and are they individually identical with those of the last diploid or pre-meiotic division? (3) Do they conjugate side by side (parasynapsis, parasyndesis), end to end (telosynapsis, metasyndesis) or in both ways? (4) Does synapsis lead to partial or complete fusion of the conjugating elements to form 'Zygosomes' or 'Mixochromosomes,' or are they subsequently disjoined by a 'reduction division'? Upon these questions depends our answer to a fifth and still more important question, namely, (5) Can the Mendelian segregation of unit factors be explained by the phenomena of synapsis and reduction?"

In *Bos taurus*, however, it is impossible to consider these whole questions of synapsis and reduction. As already described, in the telophase of the ultimate spermatogonia the chromosomes do not fuse but remain for a while scattered throughout the nucleus. In these cells thirty or more chromosomes can be counted. In postsynapsis (pachytene stage) the spiremes appear in about half the original number. These conditions clearly indicate that in the presynapsis the chromosomes are in the diploid number and conjugate during the synaptene stage.

It is difficult to determine whether the conjugation of chromosomes takes place by parasynapsis (side by side conjugation) or telosynapsis (end to end conjugation). In the horse, from many phenomena, we have reached the view that the conjugation of chromatin threads probably takes place by parasynapsis. But in cattle there is no evidence of parasynapsis taking place at all. During the synaptene stage a longitudinal duality of the spiremes, as that seen in the horse, is almost absent or, if occasionally present, is due either to an accidental parallelism, or to a longitudinal split comparable to that of the diploid prophase. But as stated above, the observation of the preparations

compelled us to admit the following facts: 1. In the early leptotene stage some of the chromosomes already begin to unite with their free ends. 2. Sometimes in these chromatin threads a longitudinal split appears, but in the synaptene stage the conjugated point of two chromosomes can not be seen.

JORDAN ('11) in opossum has indicated that in the synaptene stage (Fig. 16) most of the loops are nearly the height of the nuclear diameter, and the summits of the loops are marked by more compact chromatic knobs, the point of union of two threads. In cattle such a conjugated point of chromosomes can not be seen during the synapsis and the pachytene stage, but a transverse constriction of the chromosomes is found at the late prophase. This constriction probably represents the conjugated ends of the univalent chromosomes.



Text-fig. 1. Diagrammatic representation of synapsis and reduction of chromosome.

a, synaptene stage; b, pachytene stage; c, diplotene stage; d, late prophase; e, metaphase of the first reduction division.

separated along the point of conjugation in the reduction division.

From the acceptance of occurrence of telosynapsis, it is difficult to prove the rapid thickening of the spiremes in the pachytene stage, but this difficulty may be interpreted as follows: The thickening of the spiremes may probably be due to the fact that in the synaptene stage the spiremes as well as the nucleus grow very rapidly compared with the growth in other stages.

The longitudinal splits of chromosomes which appear during the late prophase of the first spermatocyte are apparently the same as those of the spermatogonial chromosomes. If the nature of the longitudinal split of the primary spermatocyte is entirely the same as that of the spermatogonium, then this longitudinal split is comparable to that of the somatic chromosome, and the chromosome united in the synaptic period must be separated in the first division. The first division is, therefore, a reducing division.

From the above data it is conceivable that conjugation of the chromosomes occurs by telosynapsis (end to end conjugation) during the synaptene stage, and the bivalent chromosomes thus formed may be

## VI. Summary.

1. In the testes of embryos and quite young animals the spermatogonia are divided by amitosis, and in such young individuals amitosis occurs more frequently than mitosis. Judging from their nuclear organizations and other structures, it is evident that the cells produced by amitosis are degenerating, being used as nutritive materials by the germ cells.

2. The resting nuclei in both the spermatogonial generations, the penultimate and the ultimate spermatogonia, usually contain one large nucleolus and a small chromatin mass.

3. In the spermatogonia the number of chromosomes may be counted as thirty three. These vary considerably in size and form, but are found to be in pairs. Each chromosome simultaneously divides into two portions along the longitudinal split which first appeared in the spireme stage; no special chromosome with different behavior is to be seen among them.

4. In the telophase of the ultimate spermatogonia the chromosomes are not fused, and thus it is possible to make out individual chromosomes in which the longitudinal split is rarely found. Subsequently the chromosomes become lengthened to the leptotene threads.

5. Conjugation of chromosomes probably takes place by telosynapsis during the synaptene stage. In this stage the leptotene threads converge towards one side of the nucleus, leaving a clear space on the other side.

6. During the final prophase the longitudinal split and transverse constriction of the chromosomes are found.

7. The chromosomes are divided along the constricted point in the first reduction division which is the reducing division.

8. In the second reduction division all the chromosomes become so placed that the line of the longitudinal split coincides with the equatorial plane, and along this line all the chromosomes as well as the accessory ones are divided at the same time, thus it is simply an equation-division.

9. In the spermatogonia and the spermatocyte the centrosome is so minute that it can not be distinguished from the other granules. The changes of the centrosome during the formation of the spermatozoa are considerably different from those observed in the horse. The centrosome

situated at the posterior part of the cell becomes now divided into two, and these lie side-by-side, being connected by a thick filament. The two centrosomes thus formed move apart from each other and come to be placed in such a way that the one lies close to the nuclear membrane, the other at some distance from it, and thus anterior and posterior centrosomes are produced. The latter send out a fine filament backwards towards the surface of the cell which later becomes the axial filament.

10. The chromosome nucleolus or the accessory chromosome can be traced throughout the growth stage and the reduction division. The behavior of this chromosome is quite similar to that of the horse, but it can not be distinguished in the resting stage of the secondary spermatocyte and in the spermatid.

11. The idiosome appears as a cytoplasmic body in the growth stage, and during the formation of the spermatozoa it becomes more and more conspicuous, till it assumes a small spheric body and comes to be situated in a depression at the anterior part of the nucleus. It seems to have no connection with the centrosome.

12. The mitochondrial granules make their appearance abundantly during the growth stage. Their behavior is quite similar to those of the horse.

13. Second pairing of chromosomes is not found, but as in the case of the horse incomplete fusion of the chromosomes is seen to occur, in such a case occasionally nine or ten chromosomes are counted.

14. The chromatoid corpuscle can not be found, but during the reduction division a small spheric body stained faintly with iron-haematoxylin rarely appears in the cytoplasm, which however could not be observed in all the other stages.

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#### EXPLANATION OF PLATES.

The figures were drawn with the aid of a camera lucida. Figs. 22—29, 34—38 and 46—98 were drawn with a Zeiss 1/12 objective and compensating ocular 12, Figs. 3—21, 30—33 and 39—45 with a Zeiss 1/12 objective and a Zeiss 4 ocular, Figs. 1, 2 with a Zeiss D objective and a Zeiss 4 ocular.

#### PLATE XIV.

- Fig. 1. Section of testis of an embryo, showing differentiation of spermatogonia.
- Fig. 2. Section of testis of a quite young animal, showing spermatogonium and amitotic division.
- Fig. 3. Section of testis of a quite young animal.
- Fig. 4. Spermatogonium in the earliest generation.
- Fig. 5. Spermatogonium; the nucleoli break into small pieces.
- Fig. 6. Spermatogonium which contains two nucleoli.
- Fig. 7. Spermatogonium showing division of nucleoli.
- Figs. 8, 9. Early stage of amitotic division, showing irregular outline of nuclei.
- Fig. 10. Amitotic division of a spermatogonium, showing fission of nuclear wall and a sphere.
- Figs. 11, 12. Amitotic division.
- Figs. 13—17. Multinuclear cells produced by amitosis.
- Figs. 16, 18. Amitotic division, showing chromatin spiremes.
- Fig. 19. Resting spermatogonium in the earliest generation.
- Figs. 20, 21. Spireme stage of spermatogonia in the earliest generation.
- Figs. 22, 23. Prophase nuclei of spermatogonia in the earliest generation.
- Fig. 24. Prophase nucleus of a spermatogonium in the earliest generation, showing longitudinal split of chromosome.
- Fig. 25. Nucleus of a spermatogonium (the earliest generation) in the late prophase.

- Fig. 26. Lateral view of metaphase of spermatogonium in the earliest generation.  
 Fig. 27. Side view of anaphase of spermatogonium in the earliest generation.  
 Fig. 28. Polar view of metaphase of spermatogonium in the earliest generation.  
 Fig. 29. Chromosomes of spermatogonium in the earliest generation, showing thirty three which consist of sixteen symmetrical pairs and one odd chromosome.  
 Fig. 30. Telophase of spermatogonium in the earliest generation.  
 Fig. 31. Spermatogonia and Sertoli-cell in testis of an adult animal.  
 Fig. 32. Penultimate spermatogonium in the resting stage.  
 Fig. 33. An ultimate spermatogonium in the early prophase.  
 Fig. 34. Nucleus of spermatogonium in the early prophase, showing formation of chromatin spiremes.  
 Fig. 35. Side view of metaphase of an ultimate spermatogonium.  
 Fig. 36. Side view of anaphase of an ultimate spermatogonium.

## PLATE XV.

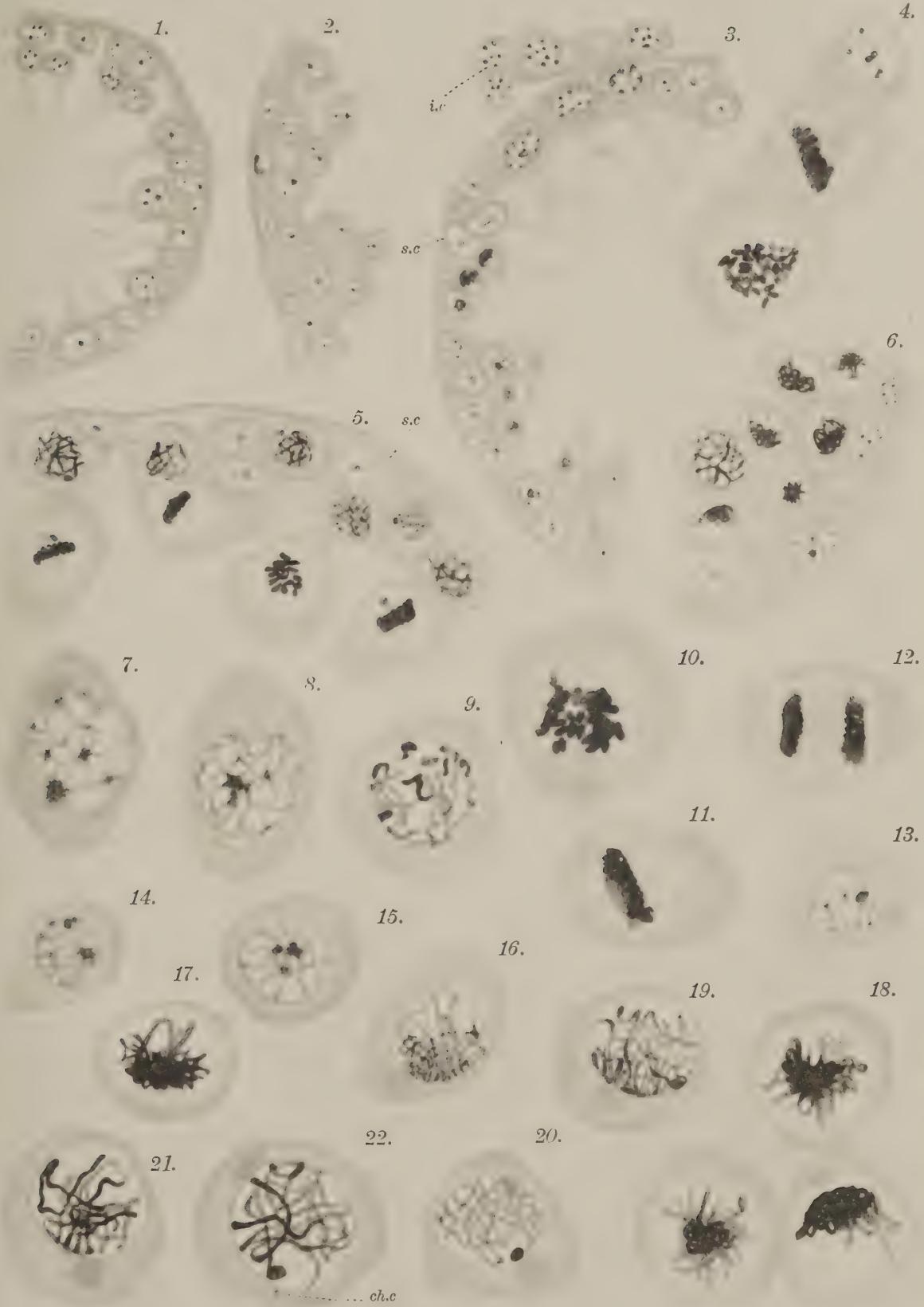
- Fig. 37. Polar view of metaphase of penultimate spermatogonium in an adult animal.  
 Fig. 38. Chromosomes of penultimate spermatogonium in an adult animal.  
 Fig. 39. Section of testis of an adult animal, showing the telophase of ultimate spermatogonia.  
 Fig. 40. Section of testis of an adult animal, showing ultimate spermatogonia, primary spermatocytes and a Sertoli-cell.  
 Fig. 41. Section of testis of an adult animal, showing synaptic period.  
 Fig. 42. Section of testis of an adult animal, showing post-synaptic stage.  
 Fig. 43. Spermatoocytes in pachytene stage.  
 Figs. 44, 45. Spermatocytes in diplotene stage.  
 Figs. 46, 47. Nuclei of telophase of ultimate spermatogonia.  
 Figs. 48—50. Nuclei of spermatocytes in leptotene stage.  
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 Fig. 58. Side view of metaphase of first reduction division.  
 Fig. 59. Side views of second divisions and polar view of telophase of a second division.  
 Fig. 60. Resting nuclei and metaphases of second spermatocytes.

## PLATE XVI.

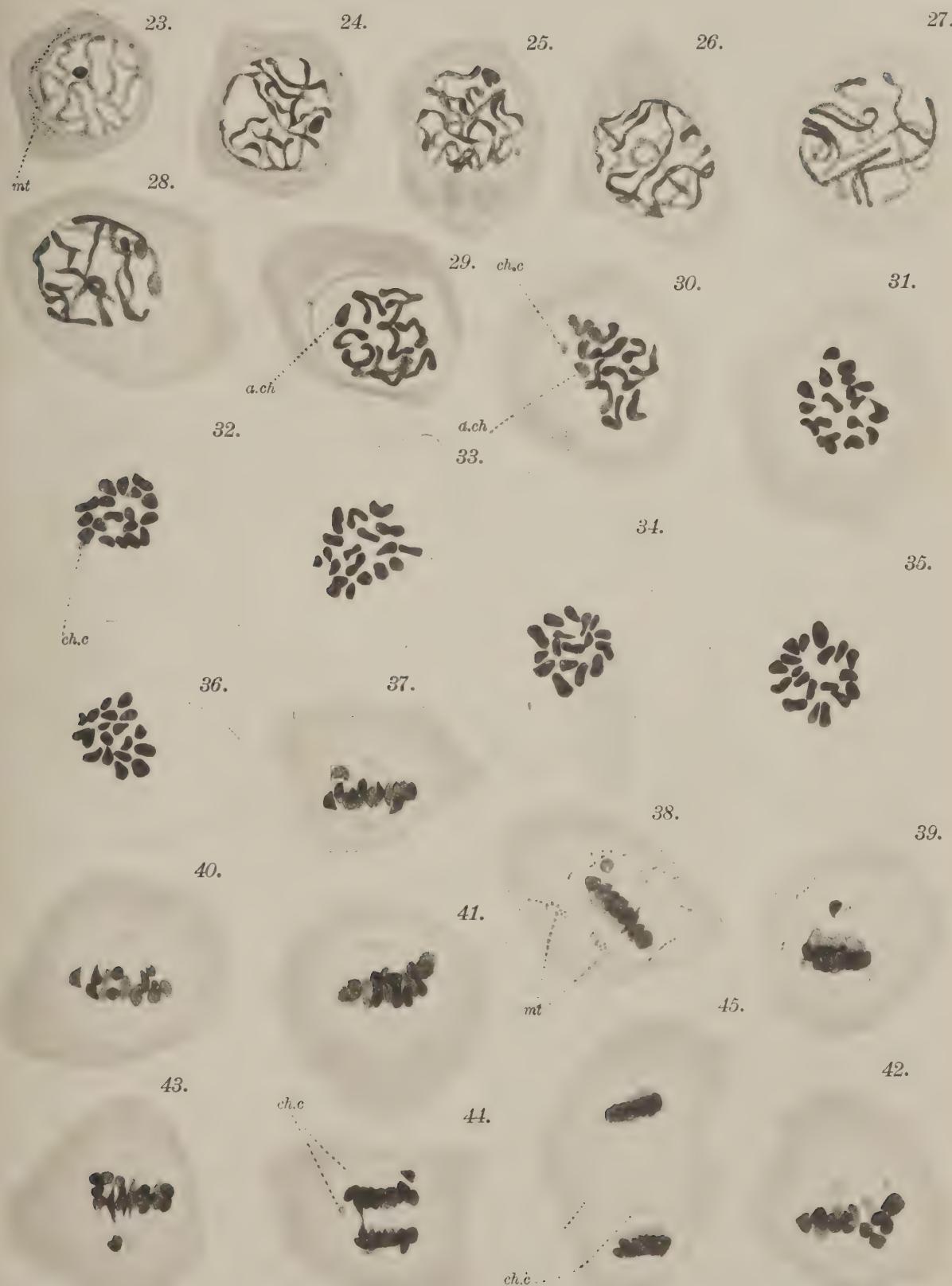
- Figs. 61, 62. Spermatocytes in diplotene stage, showing mitochondrial granules.  
 Fig. 63. Polar view of a metaphase plate of first division, showing longitudinal split of chromosome.  
 Figs. 64, 65. Polar views of metaphase plates of first division.  
 Fig. 66. Side view of metaphase of first division, showing mitochondrial granules.

- Fig. 67. Side view of metaphase of first division, showing longitudinal split of chromosome.  
Fig. 68. Side view of metaphase of first division, showing accessory chromosome.  
Fig. 69. Side view of anaphase of first division, showing accessory chromosome.  
Fig. 70. Side view of late anaphase of first division.  
Fig. 71. Telophase of first division, showing mitochondrial granules.  
Fig. 72. Telophase of first division.  
Fig. 73. A secondary spermatocyte in resting stage, showing mitochondrial granules.  
Fig. 74. A resting secondary spermatocyte; idiozome and mitochondria appear.  
Fig. 75. Polar view of metaphase of second division; a faintly stained cytoplasmic body appears near cell wall.  
Fig. 76. Polar view of metaphase of second division; accessory chromosome can be seen.  
Fig. 77. Side view of metaphase of second division.  
Fig. 78. Late anaphase of second division.  
Figs. 79—82. Telophases of second division.  
Figs. 83, 84. Late telophases of second division.  
Fig. 85. A Spermatid; mitochondrial granules gather at the posterior part of cell.  
Figs. 86, 88. Spermatids; idiosome is clearly seen.  
Fig. 87. Spermatid; centrosomes can be seen.  
Fig. 89. Spermatid; idiosome begins to migrate to the anterior wall of nucleus.  
Fig. 90. Spermatid; nucleus gradually migrates to cell wall.  
Fig. 91. Spermatid; mitochondria gather around centrosomes.  
Fig. 92. Spermatid; idiosome become fixed as a small spheric body at a depression of nuclear wall.  
Figs. 93—96. Final development of spermatozoa; dumb-bell shaped centrosomes, axial filament and a mass of mitochondria can be seen.  
Figs. 97, 98. Mature spermatozoa.
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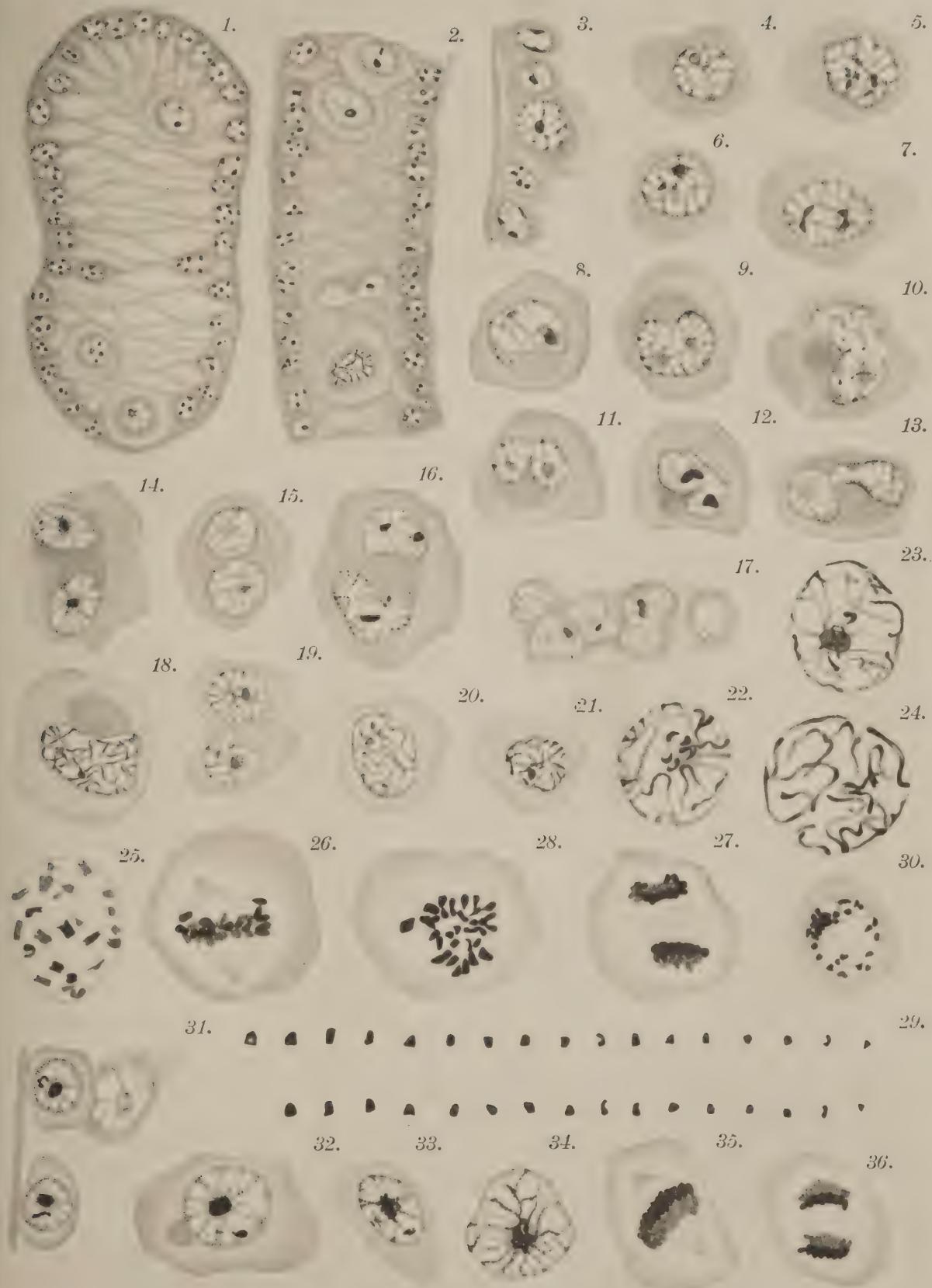
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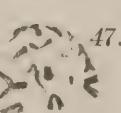
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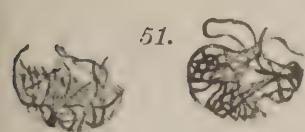
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